QK1
.A4757
v.1

Annals of the Missouri Botanical Garden

2001

Volume 88
Number 1

Volume 88, Number 1
Winter 2001

Annals of the
Missouri Botanical Garden

The Annals, published quarterly, contains papers, primarily in systematic botany, contributed from the Missouri Botanical Garden, St. Louis. Papers originating outside the Garden will also be accepted. All manuscripts are peer-reviewed by qualified, independent reviewers. Authors should write the Managing Editor for information concerning arrangements for publishing in the ANNALS. Instructions to Authors are printed in the back of the last issue of each volume and are also available online at www.mobot.org/mbgpress.

Editorial Committee

Victoria C. Hollowell
Editor,
Missouri Botanical Garden

Amy Scheuler McPherson
Managing Editor,
Missouri Botanical Garden

Diana Gunter
Associate Editor,
Missouri Botanical Garden

Aida Kadunic
Senior Secretary

Barbara Mack
Administrative Assistant

Ihsan A. Al-Shehbaz
Missouri Botanical Garden

Gerrit Davidse
Missouri Botanical Garden

Roy E. Gereau
Missouri Botanical Garden

Peter Goldblatt
Missouri Botanical Garden

Gordon McPherson
Missouri Botanical Garden

P. Mick Richardson
Missouri Botanical Garden

Henk van der Werff
Missouri Botanical Garden

For subscription information contact ANNALS OF THE MISSOURI GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897. Subscription price for 2001 is $135 per volume U.S., $145 Canada & Mexico, $170 all other countries. Four issues per volume. The journal *Novon* is included in the subscription price of the ANNALS.

annals@mobot.org (editorial queries)
http://www.mobot.org/mbgpress

© Missouri Botanical Garden 2001

THE ANNALS OF THE MISSOURI BOTANICAL GARDEN (ISSN 0026-6493) is published quarterly by the Missouri Botanical Garden, 2345 Tower Grove Avenue, St. Louis, MO 63110. Periodicals postage paid at St. Louis, MO and additional mailing offices. POSTMASTER: Send address changes to ANNALS OF THE MISSOURI BOTANICAL GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897.

The mission of the Missouri Botanical Garden is to discover and share knowledge about plants and their environment, in order to preserve and enrich life.

♾ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

Volume 88
Number 1
2001

Annals
of the
Missouri
Botanical
Garden

COEVOLUTION: BIOLOGICAL OBSERVATIONS FROM YUCCA MOTHS TO MOLECULES, AN INTRODUCTION[1]

Victoria C. Hollowell[2] *and*
P. Mick Richardson[2]

Abstract

Mutualisms between plants and animals have been observed since the nineteenth century with George Engelmann's 1872 note of the nocturnal visits of yucca moths to *Yucca*. Not until 1964 were such close ecological associations termed "coevolution" by Ehrlich and Raven. Since then, these mutualistic relationships have been seen to extend beyond plant-pollinator interactions. Examples are now known to occur between fungi and flowering plants, among marine algae, and even for the enteric bacteria of phloem-feeding insects. The 46th Annual Systematics Symposium held at the Missouri Botanical Garden in 1999 presented modern coevolution research using methodologies moving from field observation into molecular analysis.

Key words: coevolution, Engelmann, *Yucca*, mutualism, pollinator.

The ecological intimacy between flowering plants and their pollinating insects has long fascinated botanists. As proof of this, scientific study of these mutualisms and their coevolution has appeared in the literature since the 19th century. Declaring that "nocturnal insects are the agents," George Engelmann (1872a: 33, 1872b) first remarked on visits of yucca moths to *Yucca* in correspondence to the *Bulletin of the Torrey Botanical Club* (Crosby, 1974). In 1874, Engelmann detailed the close association between moth and plant in a paper for the *Transactions of the St. Louis Academy of Sciences.* Several other associates of the Missouri Botanical Garden then continued his studies. In reporting his own observations for plant-insect interactions, Charles V. Riley remarked that "this perfect adaptation and adjustment have been brought about by slow degrees through the long course of ages" (1892: 126). Garden Director (1889–1912) William Trelease continued this traditional interest in coadaptation with a series of comments in the Garden's *Annual Reports* (1892, 1893, 1894, 1898, 1902, 1907, etc.).

Thus, the intertwined natural history of plants

[1] This and the four articles that follow it are the proceedings of the 46th Annual Systematics Symposium of the Missouri Botanical Garden, *Coevolution*. The symposium was held 8–9 October 1999.

The symposium was supported in part by the National Science Foundation under grant number DEB-9420140. We thank Marshall R. Crosby and Wallace D. Dawson for their insightful reviews of this introduction.

[2] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A.

and insects has been observed by biologists for over a century. But ecological definition of the relationship only came in 1964 when Paul Ehrlich and Peter Raven coined the term "coevolution" in their classic *Evolution* paper, but shifted the focus from moths to butterflies as pollinators. As further evidence of our scientific fascination with evolution in tandem, botanists have used the specific term "coevolution" in either the title or abstract of over 77 articles in the *Annals of the Missouri Botanical Garden* alone in the 36 intervening years since 1964.

An earlier Garden symposium (20th) also focused on "Plant-Animal Coevolution" (see its 1974 introduction by Gerrit Davidse). At that symposium a young Al Gentry presented his dissertation research on coevolutionary patterns in Bignoniaceae, and William Ramírez B. reported on the textbook example of fig wasps and their reciprocal exploitation of fig synconia. It was also in 1974 that Edward Ayensu commented on bat activity in western Africa, and in general, the symposium emphasis fell on pollination and fruit dispersal at the level of the individual genus or family. Raymond Heithaus (1974) examined the effects of Costa Rican pollinators on lowland community structure demonstrating that the diversity of floral visitors and floral diversity were positively linked. Across a diversity of communities, Alan Covich (1974) looked at the energetic costs of foraging behaviors by pollinators, as well as the impact of herbivory on selection.

A quarter century later, the 46th Annual Systematics Symposium (1999) provided an updated perspective on coevolution where additional model systems and methodologies uncontemplated earlier were considered. Again held in St. Louis, the 1999 symposium saw its presentations range across phylogenetic distances as wide as kingdoms and considered profound coevolutionary dependencies down to the intracellular level. Olle Pellmyr (Vanderbilt University) gave a modern interpretation of the classic yucca–yucca moth interactions. He documented that other moths may cheat at the mutualism with yucca and explored the ecological and phylogenetic implications of these corollary interactions. Brian Farrell (Harvard University) examined the coevolving interactions between plants and phytophagous insects and extended his observations into a range of other plant and animal pathogens. How does selection act to stabilize this evolutionary change as organisms increasingly track one another? Host use and host tissue specificities affect the dynamics of population genetics. Even the taxonomic affiliations of the host organism may indicate how labile or conservative shifts will be with respect to the underlying genetic architecture. Beyond plants and pollinators, participants in the 1999 Coevolution symposium drew from diverse examples, ranging from fungi, both endo- and ectomycorrhizal, to marine red algae with their adelpho- and alloparasites. For these red algae, their mutualistic intimates arise from within their own ranks, from other red algae as shown by Lynda Goff (University of California at Santa Cruz). Morphological clues are few in these algae, but host preference is an exacting one, beginning with the injection of parasite nuclei and mitochondria into hosts. In this symposium, molecular analysis supplants past phenomenological observations of pale moths on summer evenings in probing relationships between codependent organisms.

Two coevolutionary examples presented at the 1999 symposium derive from fungi and progress from the macro- to microscopic level. Roy Halling (New York Botanical Garden) dates the phenomenon in fungi to the Devonian when endomycorrhizae first appear in the fossil record. Soon after, ectomycorrhizal Homobasidiomycota (mushrooms, truffles, and puffball fungi) also formed mutualistic associations with vascular plants during their antediluvian march onto land. Suilloid Boletaceae radiated concomitantly with the progenitors of modern conifers, forming close relationships, both ectomycorrhizal and systematic, over millions of years. Ectomycorrhizae occur on a global scale, exhibiting an association with seasonal habitats. Their plant hosts include pines and oaks, and over a dozen other plant families. Ectomycorrhizal associations are now known from Myrtaceae and Dipterocarpaceae, and have been observed even among caesalpinoid legumes. Spanning continents as they do, Halling's systematic investigations of ectomycorrhizae reveal relictual disjunct distributions. Where and what macrofungi now occur can be used to address the biogeographic distribution of their associated phanerogams through time.

Mutualisms have coevolved at the intracellular level as revealed by Chris Schardl (University of Kentucky at Lexington) for fungal endophytes of cool-season grasses of Pooideae, which may then benefit from increased drought tolerance. Within Clavicipitaceae of Ascomycota, he employed molecular tools to examine *Epichloë* species and their asexual relatives. These fungal symbionts transmit themselves among grasses, plant to plant by either contagious horizonal infection, choking down inflorescences and seed set, or by a more benign, vertical transmission carried along in the mature grains of familiar grasses such as *Festuca*, *Lolium*, and *Poa*. It is striking that his multi-gene analysis

of nuclear rDNA and beta-tubulin gene introns addresses both biological and phylogenetic species concepts as revealed by the depths of its phylogenetic signal. Those *Epichloë* species that correspond to a biological species concept appear to balance horizontal contagion and vertical seed-borne transmission, specializing to specific grass hosts and exhibiting cladistic evolution. However, those fungal species decoupling a balanced and bidirectional transmission may veer to horizontal virulence, increasing their intraspecific diversity, delaying speciation with an amplification of lineage sorting, with the ecological consequence of broadening the host base among grasses.

Another elegant example of coevolution exists for phloem-sucking insects and their endosymbionts that supply essential amino acids, which are otherwise limiting nutrients in plant sap. Nancy Moran (University of Arizona at Tucson) investigates these Proteobacteria, which reside within specialized bacteriocytes of their insect hosts and are transmitted maternally to aphid offspring. Related to enteric bacteria, aphid endosymbionts have discarded superfluous nucleic acid sequence, and consequently many functional gene pathways, and feature very small genomic sizes. For *Buchnera*, the best characterized of the aphid endosymbionts, this means its genome is only about 1/7th the size of its well-known relative *E. coli*. This mutualism is an old one, estimated at over a million years, but however long the relationship, *Buchnera* is constrained by small effective population size. Selection on the endosymbiont population exists at the level of the aphid host. This is a very small scale indeed and one in which slightly deleterious mutations accumulate to fixation in the *Buchnera* genome through genetic drift. Biosynthetic pathways for amino acids have relocated, at least twice, to plasmids in an unusual placement for bacterial genomes. Increased copy number and tandem repeats are seen on these plasmids and drive the overproduction of those amino acids required by the host insects. However coadapted these endosymbionts appear to be to their now obligate aphid hosts, the consequence appears to be an accelerated sequence evolution and biased base composition for *Buchnera* genomes.

Classic closure for the 46th Annual Systematics Symposium came with May Berenbaum's (University of Illinois at Urbana–Champaign) look at the manner in which botanists have viewed plant chemical signatures and the concomitant insect responses. Insects nibble, and plants present chemical defense. A continuum of ecological response exists for the insect, from herbivory to avoidance. Equally variable may be the selective advantage for the plant. Does its secondary phytochemistry deter or invite invertebrate companions? When does this move into the taxonomic coadaptation of flora and fauna? Well known is that lepidopterans appear more species-rich when associated with hostplants having more advanced classes of coumarins, e.g., for *Papilio* on Apiaceae (Ehrlich & Raven, 1964). Plant secondary chemistries exert further selective pressures with their chemical interactions. Methylenedioxyphenol or MDP compounds, prevalent in Apiaceae and Asteraceae, potentiate furanocoumarin activity in these plant families; MDP compounds also inhibit cytochrome P450 monooxygenases, the enzyme class in insects that detoxifies furanocoumarins. Insects specialist to these plants face this doubled toxicological challenge, and if successful gain ecological advantage for herbivory and/or colonization for themselves on these plants. Insects not preadapted do not meet the competitive challenge. In turn, similar insect faunas select for similar phytochemistries in host plants, regardless of their phylogenetic affinities.

Literature Cited

Ayensu, E. S. 1974. Plant and bat interactions in West Africa. Ann. Missouri Bot. Gard. 61: 702–727.

Covich, A. 1974. Ecological economics of foraging among coevolving animals and plants. Ann. Missouri Bot. Gard. 61: 794–805.

Crosby, M. R. 1974. George Engelmann's notes on the pollination of *Yucca*. Ann. Missouri Bot. Gard. 61: 907.

Davidse, G. 1974. Plant-animal evolution: The Twentieth Systematics Symposium: Introduction. Ann. Missouri Bot. Gard. 61: 674.

Ehrlich, P. & P. H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution 18: 586–608.

Engelmann, G. 1872a. 51. The flower of *Yucca* and its fertilization. Bull. Torrey Bot. Club 3: 33.

———. 1872b. 63. Note from Dr. Engelmann. Bull. Torrey Bot. Club 3: 37.

———. 1874. The flower of *Yucca* and its fertilization. Trans. Acad. Sci. St. Louis III: 208–211.

Gentry, A. H. 1974. Coevolutionary patterns in Central American Bignoniaceae. Ann. Missouri Bot. Gard. 61: 728–759.

Heithaus, E. R. 1974. The role of plant-pollinator interactions in determining community structure. Ann. Missouri Bot. Gard. 61: 675–691.

Ramírez B., W. 1974. Coevolution of *Ficus* and Agaonidae. Ann. Missouri Bot. Gard. 61: 770–780.

Riley, C. V. 1892. The yucca moth and yucca pollination (in Scientific Papers). Annual Rep. Missouri Bot. Gard. 1892: 99–158.

Strong, D. R. 1974. The insects of British trees: Community equilibration in ecological time. Ann. Missouri Bot. Gard. 61: 692–701.

Trelease, W. 1892. Detail illustrations of *Yucca* (in Scientific Papers; Notes and Observations). Annual Rep. Missouri Bot. Gard. 1892: 159–166.

———. 1893. Further studies of yuccas and their pol-

lination (in Scientific Papers). Annual Rep. Missouri Bot. Gard. 1893: 181–226.

———. 1894. *Yucca guatemalensis* Baker (in Scientific Papers; Notes and Observations). Annual Rep. Missouri Bot. Gard. 1894: 165–166.

———. 1898. Miscellaneous observations on Yucca (in Scientific Papers; Notes and Observations). Annual Rep. Missouri Bot. Gard. 1898: 141–146.

———. 1902. The Yucceae (in Scientific Papers). Annual Rep. Missouri Bot. Gard. 1902: 27–133.

———. 1907. Additions to the genus *Yucca* (in Scientific Papers). Annual Rep. Missouri Bot. Gard. 1907: 225–230.

ECTOMYCORRHIZAE: CO-EVOLUTION, SIGNIFICANCE, AND BIOGEOGRAPHY[1]

Roy E. Halling[2]

ABSTRACT

The fungus/plant root association known as a mycorrhizal symbiosis appears to have evolved in the Devonian. Of the two general morphological types of mycorrhizae among plant families, arbuscular mycorrhizae appear earlier in the fossil record and are more widespread than are ectomycorrhizae. The latter type of symbiosis most likely evolved with the vascular plant progenitors of modern Pinaceae in the Jurassic, but also developed with the ancestors of a dozen or so extant Angiosperm families. The significance of the ectomycorrhizal relationship is evident in that the symbionts profit by acquiring resources from their partners that would otherwise be unavailable. In natural environments where ectomycorrhizal communities exist, the symbiosis may be restricted or not depending on the particular plants and fungi involved. The presence of an obligate specificity of a particular fungus with a particular plant has a profound consequence on biogeographic distribution.

Key words: Australia, biogeography, Colombia, Costa Rica, disjunct distribution, fungi, montane tropics, mycorrhizae, symbiosis.

I address five general points regarding mycorrhizae below, with particular reference to the type known as ectomycorrhizae. These points are: (1) mycorrhizae, (2) the organisms involved, (3) co-evolution, (4) biological significance, and (5) biogeography. The discussion is not from the viewpoint of one who studies the mycorrhizal phenomenon, but rather as a mycomic systematist whose study organisms frequently form these mycorrhizal associations.

(1) MYCORRHIZAE

Mycorrhiza refers to an association/symbiosis/mutualism between plants and fungi wherein fungi colonize the cortical tissue of feeder rootlets during periods of active rootlet growth. The movement of plant-produced carbon to the fungus and fungal-acquired nutrients to the plant generally characterizes the association. Equally important are the production of growth regulators by the fungi, the protection of rootlets from pathogen invasion or heavy metal uptake, and the provision of sporocarps as food for mycophagous animals. The term "mycorrhiza," comes from the Greek "mykes + rhiza," which means *fungus-root* and was first applied to fungus-tree associations described in 1885 by A. B. Frank (1885). According to Trappe (1977a: 203), "About 95 percent of the world's present species of vascular plants belong to families that are characteristically mycorrhizal; mycorrhizae have evolved as the norm of terrestrial plant nutrition, not the exception." A clearinghouse for mycorrhiza information on the WWW can be found at: http://mycorrhiza.ag.utk.edu. Also, an updated text by Smith and Read (1997) provides an excellent overview of the subject.

Although mycorrhizae exhibit several different types of morphologies, I will address just one: the ectomycorrhiza. In terms of species numbers, the fungi predominately involved in the association are Homobasidiomycota (mushrooms, puffballs, and allies), although some Ascomycota are also important ectomycorrhiza-formers (including truffles and the pervasive *Cenococcum*). Among other features of the Homobasidiomycota, the spore-producing cell of these fungi, the non-septate basidium, is a hallmark of the group. The diagnostic feature of an ectomycorrhiza is the presence of hyphae between the root cortical cells producing a netlike structure called the Hartig net. By definition, ectomycorrhizae have a sheath, or mantle, of fungal tissue that

[1] Much of the work has been supported by the National Science Foundation (BSR 86-00424) and by the Office of Forestry, Environment and Natural Resources, Bureau of Science and Technology, of the U.S. Agency for International Development in NSF Grant No. DEB-9300798. The advice and aid of many interested parties in Latin America made much of the fieldwork possible. I am particularly grateful to Gregory Mueller, Field Museum of Natural History, for his continued collaboration over the past decade. Finally, I want to thank the director and staff of the Missouri Botanical Garden for providing the opportunity to present some of this material in oral form at their annual systematics symposium.

[2] Institute of Systematic Botany, The New York Botanical Garden, Bronx, New York 10458-5126, U.S.A.

may completely cover the absorbing root (typically the fine feeder roots). The mantle can vary widely in thickness, color, and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often affects fine-root morphology, resulting in bifurcate, pinnate to irregular, or coralloid branching. Sometimes hyphal strands extend into the soil from the mantle. Often hyphae aggregate to form rhizomorph-like strands that may be visible to the unaided eye. The internal portion of these strands can differentiate into tubelike structures specialized for long-distance transport of nutrients and water. A color atlas of specific ectomycorrhizal morphologies can be found in Agerer (1987–1999). In some associations, persistent intracellular penetration of the cortical cells occurs in which case the association is called an ectendomycorrhiza. One form of this type of mycorrhiza known as "arbutoid" occurs with some members of the Ericaceae and has characteristics of both ectomycorrhizae and endomycorrhizae. Intracellular penetration occurs in the root cortex, a mantle forms, and a Hartig net is present. These associations are found on *Arbutus* (e.g., Pacific madrone), *Arctostaphylos* (e.g., bearberry and manzanitas), and several species of the Pyrolaceae. In the monotropoid subtype, the fungi colonize achlorophyllous (lacking chlorophyll) plants in the Monotropaceae (e.g., Indian pipe and Snow plant), producing the Hartig net and mantle.

(2) The Organisms Involved

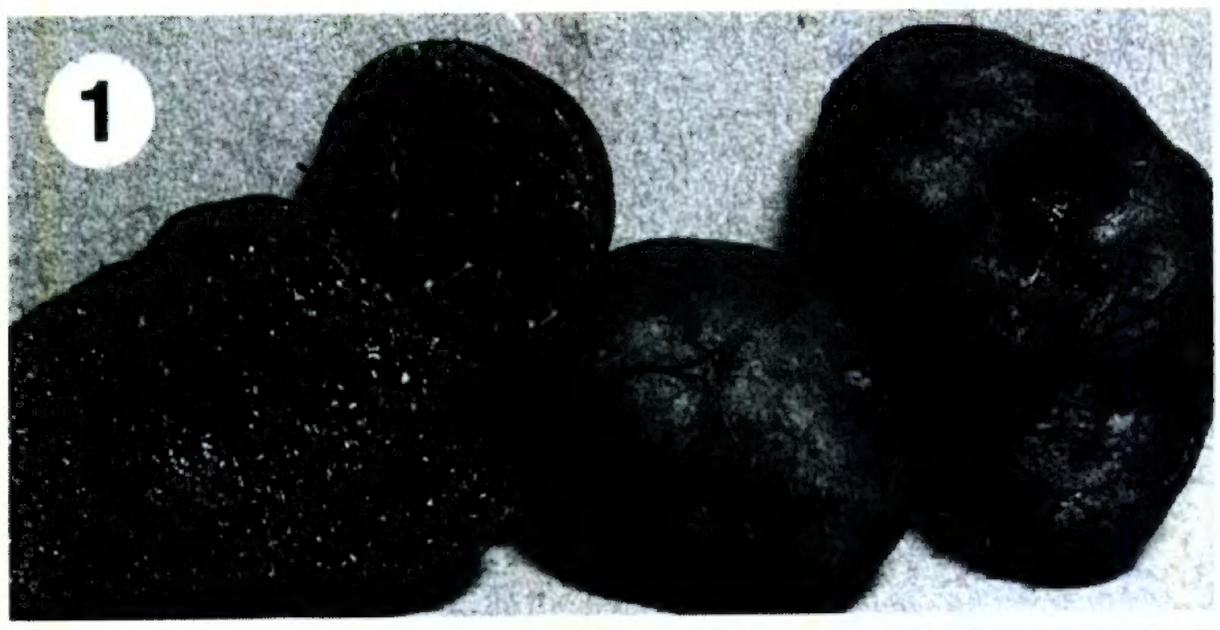
Ectomycorrhizae are found on certain woody plants. They are worldwide in distribution and are particularly adapted to habitats with marked seasonal changes, especially where periodic flushes of nutrients occur in the soil. One estimate (Molina et al., 1992) states that between 5000 and 6000 fungal species, belonging primarily to the Homobasidiomycota (at least 25 families according to Miller, 1982), and fewer to the Ascomycota (± 9 families), are known to form ectomycorrhizae. Many of these fungi produce mushrooms, puffballs, truffles, and false truffles on the forest floor. Some fungi are restricted to a single plant species, like *Suillus grevilleii* (Klotzsch) Singer on larch, *Truncocolumella citrina* Zeller on Douglas fir, or *Suillus spraguei* (Berk. & Curt.) Kuntze on eastern white pine. In contrast, others have a very broad host range, such as *Pisolithus tinctorius* (Pers.) Coker & Couch, which will form ectomycorrhiza with more than 40 tree species belonging to at least eight genera. We know that a single plant, or plant species, usually forms ectomycorrhizae simultaneously with many different fungi at the same time in the same place, and a succession of different fungi occurs over the lifetime of a plant. In the case of Douglas fir, an important timber resource in the western U.S.A., mycorrhizists at the U.S.D.A. Forestry Laboratory in Corvallis, Oregon (Molina et al., 1992) estimate that around 2500 fungi associate with that tree, some exclusively, some not.

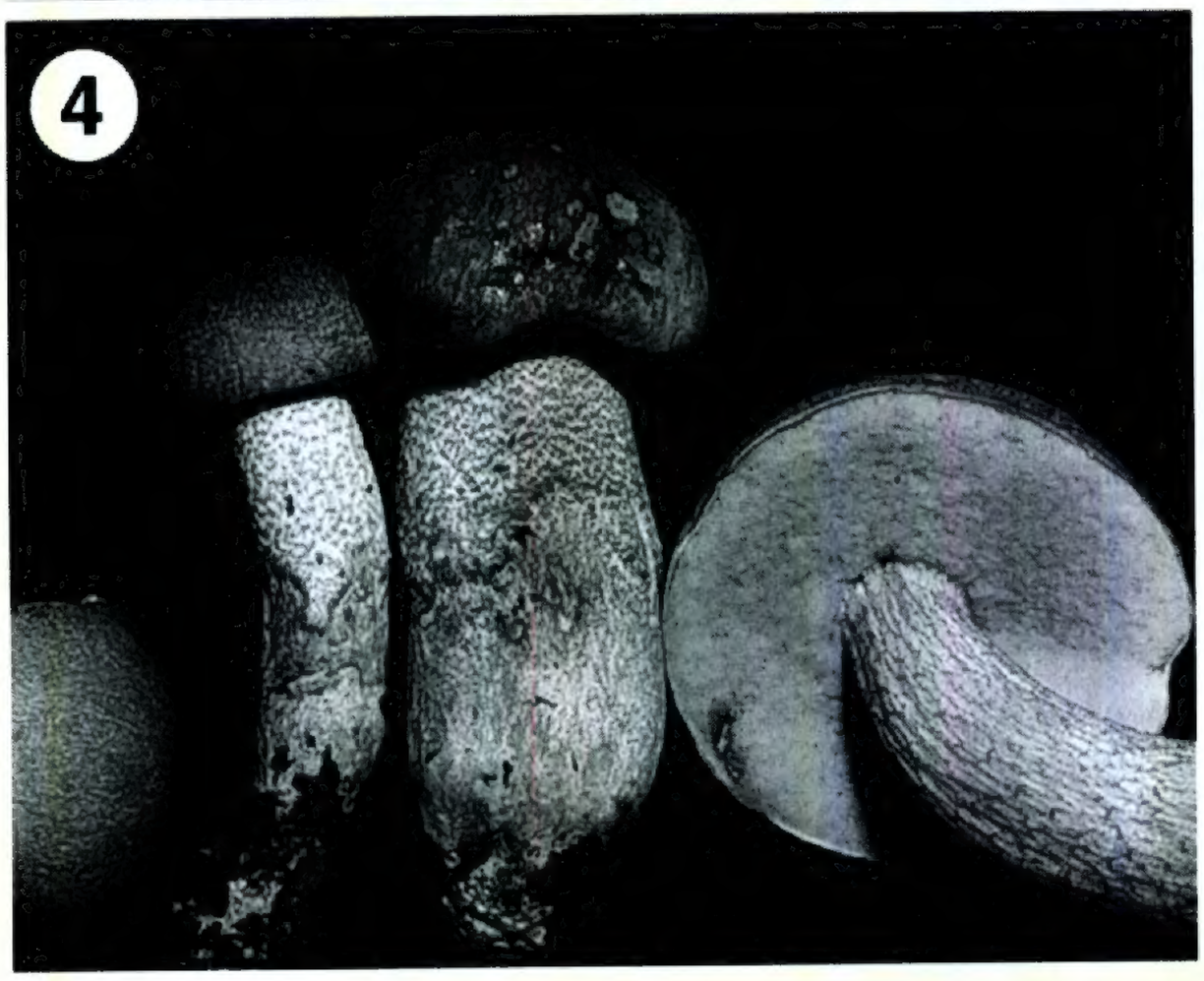
Only about 3% of phanerogams form ectomycorrhizae (Meyer, 1973). While this number is relatively small in terms of overall numbers of vascular plants, the global importance is tremendous because of their disproportionate occupancy of the land surface and their economic value as timber sources. Most of the plants, such as members of the Pinaceae and Fagaceae, dominate forests outside of the tropics along with eucalypts and other Myrtaceae in temperate and tropical Australasia, and dipterocarps, which dominate forests of tropical Indo-malaya. Considerable recent work shows that many caesalpinoid legumes are ectomycorrhizal on nutrient-poor soils in the lowland tropics of Africa and South America and will form canopy-dominant stands (Alexander, 1989; T. Henckel, pers. comm.) in which they do not support nitrogen-fixing bacteria. Also, in the montane Neotropics, recent north temperate immigrants such as oak and alder occupy areas in which they are the dominate canopies (Kappelle, 1996). It is of interest to note that the ectomycorrhizal fungal genera associated with these trees are also of north temperate origin (see below).

(3) Co-Evolution

It has been postulated that fungi were critical in the transition of ancient plants from aquatic habitats to land by enhancing water and nutrient uptake as well as decomposition of substrates and development of soils in rather harsh landscapes (Malloch et al., 1980; Bougher & Tommerup, 1996). The earliest fossil mycorrhiza dates from ~410 mya, suggesting that the mycorrhizal way of life was well established by the Devonian.

Trappe (1977b) and Trappe et al. (2000) postulated that the present disjunct distribution of truffle-like fungi in Europe and North America (Figs. 1, 2) that sporulate underground (and depend on animals for dispersal) is best explained by their widespread occurrence throughout Laurasia prior to the tectonic events that broke up the animals' overland migration routes some 50 mya. While it is commonly accepted that aboveground forms (mushroom-producing agarics) are ancestral to those that produce truffle-like sporocarps (e.g., Thiers, 1984; Bruns et al., 1989; Mueller & Pine, 1994), those





Figures 1–8. Habits of false-truffles and mushrooms. —1. *Alpova* sp. (×1). —2. *Hydnotrya tulasnei* (×0.75). —3. *Pulveroboletus ravenelii* (×1). —4. *Tylopilus chromapes* (×0.5). —5. *Tylopilus queenslandianus* (×0.75). —6. *Lactarius costaricensis* (×0.75). —7. *Amanita conara*, nom. prov. (×0.3). —8. *Amanita costaricensis*, nom. prov. (×0.3).

mushroom-forming fungi that form ectomycorrhizae would have had to originate at some point before the 50 myr mark. Based on molecular clock dating of nuclear small-subunit ribosomal DNA sequences, Berbee and Taylor (1993) suggested that divergence of the Homobasidiomycota occurred in the Triassic, ~220 mya ± 50 myrs. If the Pinaceae (95% of which form ectomycorrhizae fide Newman & Reddell, 1987) are assumed to be monophyletic and plesiomorphically ectomycorrhizal, then the existence of *Compsostrobus*, a late Triassic fossil that is thought to be an ovulate cone of Pinaceae

(Delevoryas & Hope, 1987), could possibly indicate that ectomycorrhizal Homobasidiomycota might have arisen by ~200 mya. The oldest fossils of ectomycorrhiza are 50-myr-old associates of *Pinus* roots from the Princeton chert of British Columbia (LePage et al., 1997; Hibbett et al., 1997a). The fungal component is morphologically similar to the extant truffle-like genus *Rhizopogon*, but the true identity remains unknown because of a lack of the diagnostic spore-producing stage. Ectomycorrhizal fungi probably concomitantly diversified in the Jurassic, when ectomycorrhizal Gymnosperms were globally establishing a presence (before the break-up of Pangaea). The subsequent diversification of Angiosperms probably catalyzed a boost in the diversity of ectomycorrhizal fungi soon after these plants appeared in the Cretaceous (146–65 mya) (Raven & Axelrod, 1974; Talent, 1984; Truswell et al., 1987).

The recent discovery and eventual description of *Archaeomarasmius*, a possible member of the Tricholomataceae fide Hibbett et al. (1997a) from mid-Cretaceous New Jersey amber, suggest, at the very least, that an extant mushroom morphology existed at a minimum of 90–94 mya. Taking that date and the probable date of another fossil agaric, *Coprinites* (15–30 mya), and dated attine ant fossils (15–30 mya) (both in Dominican amber) and mapping these on the attine/fungus molecular co-phylogeny produced by Chapela et al. (1994), Hibbett et al. (1997a) hypothesized minimum divergence dates for nodes of that phylogeny. They suggested that the primarily ectomycorrhizal families (basal to the rest of the phylogeny) Boletaceae and Russulaceae could date from at least the mid-Cretaceous (90 mya) when Angiosperms appeared. According to Hibbett and Thorn (2000), by mapping ectomycorrhizae as a feature on a molecular phylogeny of Homobasidiomycota (Hibbett et al., 1997b), it appears possible that the ability to form ectomycorrhizae could have arisen eight different times. However, they indicate that this inference is weak due to insufficient sampling of taxa.

Suillus s.l. and *Rhizopogon* are the two largest genera in the suilloid group of mushrooms and false-truffles (of the Boletaceae). According to Bruns et al. (pers. comm.), most larger clades and grades within these genera, and the radiation at large, are associated with subgroups of white pine or red pine, Douglas fir, or larch, but these specific associations are convergent. They estimate the timing of the suilloid radiation at 60 to 35 mya (Eocene), which is recent relative to the origin of genera in the Pinaceae (late Triassic). This timing difference and the convergence of host-pattern argues against co-cladogenesis. Nevertheless, host fidelity within the suilloids is high over evolutionary time scales, and host-switching events are rare and usually restricted to a suite of closely related plant genera. However, speciation of the fungi within a taxonomic suite of plant associates is common. In North America, there are ± 10 spp. of *Suillus* [e.g., *S. serotinus* (Frost) Kretzer & Bruns] associated only with *Larix*, for example. At the extreme, as I mentioned before, there are estimates that there may be over 2500 species of ectomycorrhizal fungi associated with Douglas fir (Molina et al., 1992). Some fungi will be found only with that tree (e.g., *Truncocolumella citrina*), while others can associate with members of other genera and families of plants.

The fungi of the Monotropaceae, a plant family consisting of achlorophyllous epiparasites, also form ectomycorrhizae with nearby coniferous trees and thereby form a link through which carbon and other nutrients can flow from the autotrophic host plant to the heterotrophic, epiparasitic plant. Using DNA fingerprinting techniques, Cullings et al. (1996) demonstrated in the monotropes that *Sarcodes sanguinea* Torr. (Snow plant) appeared to be a generalist in its fungal associations, while *Monotropa uniflora* L. (Indian pipe) from widely divergent habitats associated exclusively with members of the Russulaceae. *Monotropa hypopithys* L. was more specialized in associating with *Suillus* and/or *Rhizopogon*. *Pterospora andromeda* Nutt. from several geographic regions was at the extreme, forming mycorrhizae only with *Rhizopogon subcaerulescens* A. H. Smith, a false-truffle. The authors (Cullings et al., 1996: 64) ". . . argue that the specificity resulted from a one-sided selection on these parasitic plants to increase their fitness by optimizing carbon allocation, rather than from co-evolution in the sense of reciprocal selection." What are the benefits of such a specialization that outweighs the loss of opportunity provided by a generalist strategy? They hypothesize that the suilloid fungi, in specialization with Pinaceae, have become more efficient at obtaining carbon from their partners, and that the most widespread monotropes in those pine ecosytems have in turn specialized on the most efficient conduit of fixed carbon.

Largent et al. (1980a) showed that *Amanita gemmata* (Fr.) Gillet formed ectendomycorrhizae with manzanitas (*Arctostaphylos* spp.) and ectomycorrhizae with lodgepole pine (*Pinus contorta* Dougl. ex Loud.) in two different populations. In another study, Largent et al. (1980b) demonstrated the variability of type of ectomycorrhizae formed by species of Ericales. Some of each mycorrhizal type:

ecto, arbutoid, or ericoid could be found on the same plant species, madrone (*Arbutus menziesii* Pursh.), for example.

(4) Biological Significance

Pirozynski and Malloch (1975: 154) speculated that the appearance of plants on land could only have happened through the establishment of a mycorrhizal symbiosis; it had to be a new lifestyle, a way uniquely capable of conquering a hostile habitat, ". . . a biological innovation on a scale equal to or surpassing that responsible for the Cambrian 'explosion'." This association allowed for increased efficiency in nitrogen and phosphorus uptake and was necessary for the exploitation of new habitats and development of more complex tissue systems (Pirozynski & Malloch, 1975). According to Malloch et al. (1980, and references cited therein), it seems clear that ectomycorrhizae conferred a selective advantage on the plants, making it possible for them to occur in pure or nearly pure stands in marginal habitats (such as at high elevations or latitudes, very poor soils, and/or areas with dramatic seasonal climatic shifts). Clearly, ectomycorrhizal associates have selective value in extreme environments because of their direct role in breaking down leaf litter and a more specialized and controlled recycling of nutrients back to the plants (Malloch et al., 1980, and references cited therein).

By effectively colonizing a larger volume of soil than plant roots do alone, the benefits that plants accrue from mycorrhizal symbioses can be generally characterized by increased growth and yield and improved fitness. For example, it has been documented that mycorrhizae affect phosphorus nutrition in plants (Allen, 1991). The fungus benefits directly from a link to a proven carbon source (and becomes a carbon sink according to M. Allen, pers. comm.). In any case, the benefit to both associates arises primarily because mycorrhizal fungi critically link plant roots with the soil. The mycorrhizal hyphae in the soil: (1) take up mineral nutrients from the soil solution and transport those nutrients to the root; and (2) release nutrients from the litter/humus layers by the production of enzymes involved in mineralization of organic matter. In this regard, we are talking about the breakdown of cellulosic and complex lignosic compounds by the fungus to be utilized by other soil organisms or the plant. By the first mechanism, a mycorrhiza increases the effective absorptive surface area of the plant's feeder roots. In nutrient-poor or moisture-deficient soils, nutrients and water taken up by the soil hyphae can lead to improved plant growth and subsequent reproduction. As a result, mycorrhizal plants compete and tolerate environmental stresses better than non-mycorrhizal plants. These physiological benefits have been well documented in many case studies over the past several decades (Smith & Read, 1997).

In much of the northern spotted owls' range, old growth conifer forests provide relatively high densities of their preferred prey, the northern flying squirrel and the red-backed vole. Ninety percent of these rodents' diet consists of ectomycorrhizal, underground, truffle-like fungi in these large, dense forests of the Pacific Northwest (Hayes et al., 1986; Maser et al., 1978, 1985). The occurrence and benefit of mycorrhizal fungi in this forest community has been well described (Molina et al., 1992). Thus, in this situation, we see a tight, four-part harmony in which a bird species preys on rodent species that eat truffle-like mycorrhizal fungi which, in turn, are responsible for maintaining the health of Douglas fir trees whose branches and trunks provide prime roosting and nesting sites for the bird. As is often the case, charismatic macrofauna, like the owl, become the icon for conservation of unique and pristine ecosystems, yet experimental evidence verifies that ectomycorrhizal fungi are the actual keystone organisms (Amaranthus et al., 1989; Lamont, 1992; Grove & LeTacon, 1993; Read, 1993). Since the squirrel can adapt to laboratory rations and the vole cannot because of dentition, squirrel mycophagy is likely adaptive whereas the vole appears to represent co-evolution (Trappe, pers. comm.).

The previous example is not an isolated incident; small animal mycophagy has been documented for more than 20 years (Trappe & Maser, 1977; Fogel & Trappe, 1978). Similarly, there is additional documentation of similar feeding habits in less mesic environments by at least two Australian marsupials (brush tail rat kangaroo and southern brown bandicoot), which consume truffle-like fungi that form ectomycorrhizae with *Eucalyptus* (Malaczjuk et al., 1987). A comprehensive review of Australian mammal mycophagy has been extensively documented by Claridge et al. (1996). According to Andrew Claridge (pers. comm.), the Australian sooty owl consumes the mycophagous bush rat, as a core component of its diet, as well as the long-nosed bandicoot, which is notorious for consuming ectomycorrhizal fungi. Here is an analogous predator/prey/plant/fungus relationship in the Southern Hemisphere.

(5) Biogeography

While my co-workers and I have been documenting ectomycorrhizal associations and distri-

butions during the last two decades, we have noticed some distinctive patterns: (1) relictual disjunct distribution of (A) an individual species, (B) a species group, and (C) a genus; and (2) generic and specific distributions along a cline.

(1) RELICTUAL DISJUNCT DISTRIBUTIONS

(A) Based on its current distribution, *Pulveroboletus ravenelii* (Berk. & Curt.) Murrill (pers. obs.) (Fig. 3), with basidiomata of variable size, exhibits a relictual disjunct distribution. I would suggest that it most likely has an ancient genome with minor phenotypic change, and is a generalist in its mycorrhizal associations. It partners with Pinaceae and various dicot genera in eastern and western North America. In its southern distribution in the Americas, *P. ravenelii* associates solely with *Quercus*. It is also reported from Fagaceae forests in southeastern Asia, dipterocarps in Indo-Malaya (Corner, 1972), Myrtaceae and Casuarinaceae in Queensland, Australia (pers. obs.), but is notably absent in Europe (pers. obs.).

(B) *Tylopilus* subg. *Roseoscabra* presents a different scenario (the *T. chromapes* group fide Wolfe & Bougher, 1993). Eight species were added by those authors to the original *T. chromapes* (Frost) Smith & Thiers (Fig. 4), including one (*T. cartagoensis* Wolfe & Bougher) from a *Quercus* forest in Costa Rica and two [*T. queenslandianus* Wolfe & Bougher (Fig. 5) and *T. palumanus* Wolfe & Bougher] from Myrtaceae and Casuarinaceae forests in northeastern Australia among others from east Asia (Japan and China). Wolfe and Bougher (1993) speculated that there was a Laurasian origin of the ancient genotype and subsequent migration to and speciation in Australia during Pleistocene glaciations via land bridges. Today known associates of subgenus *Roseoscabra* are Pinaceae, Fagaceae, Betulaceae, Salicaceae, Myrtaceae, Mimosaceae, and Casuarinaceae (distributions in the eastern United States, Costa Rica, Japan, and China to northeastern Australia; the subgenus is absent in Europe). Again, I hypothesize that we are probably looking at an ancient genome, a generalist mycorrhizal proclivity, but with some phenotypic change or genetic drift/allopatric speciation.

(C) Bougher et al. (1994) indicated that the obligate ectomycorrhizal mushroom *Rozites* essentially has a Gondwanan distribution. The genus seems to have most likely co-evolved with a Cretaceous fagalean complex with more genetic diversity going south (18 species known today), where it co-evolved with *Nothofagus* (Australia, New Zealand, Chile, Argentina, New Caledonia, Papua New Guinea) and/or with Myrtaceae (in Australia). Lesser diversity for *Rozites* evolved in the Northern Hemisphere (only 3 species) and occurred after the continental breakup. We know that *Nothofagus* was distributed across southern Australia when it rafted away from Antarctica, but gradually went extinct in part of its range with increasing aridity across the region known as the Nullarbor Plain. Fossils of *Nothofagus* on that continent and its existence in South America and elsewhere have been the subject of much biogeographical debate (see references cited in Bougher et al., 1994). When *Nothofagus* went extinct in southwestern Australia (known also for its enigmatic flora) in the Eocene/Pliocene, Bougher et al. (1994) speculated that the relictual *R. symeae* Bougher et al. apparently jumped ship (switched hosts) from *Nothofagus* to relictual species of *Eucalyptus*. Interestingly, and not coincidentally, there is another species, *R. fusipes* Horak & Taylor (apparently a sister taxon to *R. symeae* fide Bougher et al., 1994), associated with *Nothofagus* in New Zealand and with *Nothofagus* and *Eucalyptus* in Tasmania. In the laboratory, Bougher (1987) demonstrated pure culture synthesis of ectomycorrhizae with *Nothofagus* and *Eucalyptus* using isolates of *Descolea maculata* Bougher & Malajczuk from Western Australia eucalypt forests. In the New World, Halling (1989) and Mueller and Strack (1992) have documented similar host shifts of mushroom species from conifers in the United States to oaks in Colombia and Costa Rica.

(2) GENERIC AND SPECIFIC DISTRIBUTIONS ALONG A CLINE

In the New World montane tropics, especially Costa Rica and Colombia, Greg Mueller and I (and our collaborators) have been documenting the macrofungus diversity (e.g., Halling & Ovrebo, 1987; Halling, 1996; Halling & Mueller, 1999; Mueller, 1996; Mueller & Halling, 1995; http://www.nybg.org/bsci/res/hall/costaric.html). Although we are not the first to note affinities of montane Neotropical Agaricales with taxa in the North Temperate zone (see Moser & Horak, 1975), we do suggest co-migration of mycorrhizal communities; that is, obligate ectomycorrhizal fungal genera have migrated from the North Temperate zone with their associated phanerogams, specifically alder and oak, both of which had a North Temperate origin. Palynological studies by Hooghiemstra and Cleef (1995) around Bogotá, Colombia, suggest that oak communities have been in the northern Andes less than 350,000 years and alder less than 1 million years.

A general analysis of phenetic similarities in one family and three genera of ectomycorrhizal mushrooms showed greater (at higher taxonomic levels) or lesser (at lower taxonomic levels) affinities with North Temperate taxa of the Western Hemisphere (Mueller & Halling, 1995). These data indicate a definite north/south clinal distribution pattern in four different ways.

- North temperate to southern Colombia: *Cortinarius iodes* Berk. & Curt., *Lactarius indigo* (Schw.) Fr., *L. atroviridis* Peck, *Laccaria amethystina* Cooke, *Strobilomyces confusus* Singer. These species have the largest distribution range.
- North temperate, south to Costa Rica: *Asterophora parasitica* (Fr.) Singer, *Boletus frostii* Russell, *Lactarius psammicola* A. H. Smith, *Lactarius rimosellus* Peck, *Russula nigricans* (Bull.: Fr.) Fr., *Tylopilus eximius* (Peck) Singer. Nota bene (N.b.), *Asterophora* is an obligate parasite of the obligately ectomycorrhizal *Russula.*
- Neotropical oak endemics: *Amanita garabitoana,* nom. prov., *Laccaria gomezii* Mueller & Singer, *Lactarius costaricensis* Singer (Fig. 6), *Leccinum andinum* Halling, *Rozites colombiana* Halling & Ovrebo. N.b., these genera are widespread north temperate entities, but these particular species appear endemic to the montane Neotropics.
- Restricted endemics (currently): *Amanita conara,* nom. prov. (Fig. 7), *A. costaricensis,* nom. prov. (Fig. 8), *Boletus flavoniger* Halling et al., *Leccinum talamancae* Halling et al., *Tricholosporum violaceum* Halling & Franco-M. N.b., the genera are widespread north temperate taxa, but these species seem restricted to only a few sites in the Cordillera Talamanca of Costa Rica.

Summary

Mycorrhizal associations have been around for a long time, at least since life forms migrated to terrestrial habitats. As these new habitats or niches became available, fungal and plant partners, both symbionts together as a community, likely explored and exploited new niches for resources. Some host-specific fungi and plants have coevolved in earnest and migrated together, to a greater or lesser extent, over the millennia depending on habitat availability and the existence of potential partners. This fungus-plant union that occupies most of the terrestrial part of our planet would appear responsible for the very appearance of the earth's natural landscape.

Literature Cited

Agerer, R. (Editor). 1987–1999. Colour Atlas of Ectomycorrhizae: With Glossary. Einhorn Verlag, Schwäbisch-Gamünd. 2 vols. (loose-leaf).

Alexander, I. J. 1989. Systematics and ecology of ectomycorrhizal legumes. Advances in Legume Biology. Monogr. Syst. Bot. Missouri Bot. Gard. 29: 607–624.

Allen, M. F. 1991. The Ecology of Mycorrhizae. Cambridge Univ. Press, Cambridge.

Amaranthus, M. P., J. M. Trappe & R. J. Molina. 1989. Long-term forest productivity and the living soil. Pp. 36–52 *in* D. A. Perry et al. (editors), Maintaining the Long-term Productivity of Pacific Northwest Forest Ecosystems. Timber Press, Portland.

Berbee, M. L. & J. W. Taylor. 1993. Dating the evolutionary radiations of the true fungi. Canad. J. Bot. 71: 1114–1127.

Bougher, N. L. 1987. The Systematic Position and Ectomycorrhizal Status of the Fungal Genus *Descolea.* Ph.D. Thesis, University of Western Australia, Perth.

——— & I. C. Tommerup. 1996. Conservation significance of ectomycorrhizal fungi in Western Australia: Their co-evolution with indigenous vascular plants and mammals. Pp. 299–308 *in* S. D. Hopper et al. (editors), Gondwanan Heritage: Past, Present and Future of the Western Australian Biota. Surrey Beatty & Sons, Chipping Norton.

———, B. A. Fuhrer & E. Horak. 1994. Taxonomy and biogeography of Australian *Rozites* species mycorrhizal with *Nothofagus* and Myrtaceae. Austral. Syst. Bot. 7: 353–375.

Bruns, T. D., R. Fogel, T. J. White & J. D. Palmer. 1989. Accelerated evolution of a false-truffle from a mushroom ancestor. Nature 339: 140–142.

Chapela, I. H., S. A. Rehner, T. R. Schultz & U. G. Mueller. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. Science 266: 1691–1694.

Claridge, A. W., M. A. Castellano & J. M. Trappe. 1996. Fungi as a food resource for mammals in Australia. Pp. 239–267 *in* K. Mallett & C. Grgurinovic (editors), Fungi of Australia, Vol. 1B. Australian Biological Resources Study, CSIRO, Canberra.

Corner, E. J. H. 1972. *Boletus* in Malaysia. Government Printing Office, Singapore.

Cullings, K. W., T. M. Szaro & T. D. Bruns. 1996. Evolution of extreme specialization within a lineage of ectomycorrhizal epiparasites. Nature 379: 63–66.

Delevoryas, T. & R. C. Hope. 1987. Further observations on the late Triassic conifers *Compsostrobus neotericus* and *Voltzia andrewsii.* Rev. Palaeobot. Palynol. 51: 59–64.

Fogel, R. & J. M. Trappe. 1978. Fungus consumption (mycophagy) by small animals. Northw. Sci. 52: 1–31.

Frank, A. B. 1885. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. Deutsch. Bot. Ges. 3: 128–145.

Grove, T. S. & F. Le Tacon. 1993. Mycorrhiza in plantation forestry. Pp. 191–227 *in* I. C. Tommerup (editor), Advances in Plant Pathology, Vol. 9. Mycorrhiza Synthesis. Academic Press, London.

Halling, R. E. 1989. A synopsis of Colombian boletes. Mycotaxon 34: 93–113.

———. 1996 (1997). Boletaceae (Agaricales): Latitudinal biodiversity and biological interactions in Costa

Rica and Colombia. Revista Biol. Trop. 44: (supplement 4): 111–114.

——— & G. M. Mueller. 1999. New boletes from Costa Rica. Mycologia 91: 893–899.

——— & C. L. Ovrebo. 1987. A new species of *Rozites* from oak forests of Colombia with notes on biogeography. Mycologia 79: 674–678.

Hayes, J. P., S. P. Cross & P. W. McIntire. 1986. Seasonal variability in mycophagy by the western red-backed vole, *Clethrionomys californicus*, in southwestern Oregon. Northw. Sci. 60: 250–257.

Hibbett, D. S. & R. G. Thorn. 2000. Homobasidiomycetes. *In*: P. Lemke & D. J. McLaughlin (editors), The Mycota, Vol. VII. Systematics and Evolution. Springer-Verlag, New York (in press).

———, D. Grimaldi & M. J. Donoghue. 1997a. Fossil mushrooms from Miocene and Cretaceous ambers and the evolution of Homobasidiomycetes. Amer. J. Bot. 84: 981–991.

———, E. M. Pine, E. Langer, G. Langer & M. J. Donoghue. 1997b. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc. Natl. Acad. Sci. U.S.A. 94: 12002–12006.

Hooghiemstra, H. & A. M. Cleef. 1995. Pleistocene climatic change and environmental and generic dynamics in the north Andean montane forest and páramo. Pp. 35–49 *in* S. Churchill, H. Balslev, E. Forero & J. Luteyn (editors), Biodiversity and Conservation of Neotropical Montane Forests. New York Botanical Garden Press, Bronx.

Kappelle, M. 1996. Los Bosques de Roble (*Quercus*) de la Cordillera de Talamanca, Costa Rica: Biodiversidad, Ecología, Conservación y Desarollo. Univ. Amsterdam & Instituto de Biodiversidad.

Lamont, B. B. 1992. Functional interactions within plants—The contribution of keystone and other species to biological diversity. Pp. 95–127 *in* R. J. Hobbs (editor), Mediterranean Ecosystems in Australia. Surrey Beatty & Sons, Chipping Norton.

Largent, D. L., N. Sugihara & A. Brinitzer. 1980a. *Amanita gemmata*, a non-host specific mycorrhizal fungus of *Arctostaphylos manzanita*. Mycologia 72: 435–439.

———, ——— & G. Wishner. 1980b. Occurrence of mycorrhizae on ericaceous and pyrolaceous plants in northern California. Canad. J. Bot. 58: 2274–2279.

LePage, B. A., R. S. Currah, R. A. Stockey & G. W. Rothwell. 1997. Fossil ectomycorrhizae from the Middle Eocene. Amer. J. Bot. 84: 410–412.

Malajczuk, N., J. M. Trappe & R. Molina. 1987. Interrelationships among some ectomycorrhizal trees, hypogeous fungi and small mammals: Western Australian and northwestern American parallels. Austral. J. Ecol. 12: 53–55.

Malloch, D. W., K. A. Pirozynski & P. H. Raven. 1980. Ecological and evolutionary significance of mycorrhizal symbioses in vascular plants (A Review). Proc. Natl. Acad. Sci. U.S.A. 77: 2113–2118.

Maser, C., J. M. Trappe & R. A. Nussbaum. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. Ecology 59: 799–809.

Maser, Z., C. Maser & J. M. Trappe. 1985. Food habits of the northern flying squirrel (*Glaucomys sabrinus*) in Oregon. Canad. J. Zool. 63: 1084–1088.

Meyer, F. H. 1973. Distribution of ectomycorrhizae in native and man-made forests. Pp. 79–105 *in* G. C. Marks & T. T. Kozlowski (editors), Ectomycorrhizae. Academic Press, New York.

Miller, O. K., Jr. 1982. Taxonomy of ecto- and ectendomycorrhizal fungi. Pp. 91–101 *in* N. C. Schenck (editor), Methods and Principles of Mycorrhizal Research. Amer. Phytopathol. Soc., St. Paul, Minnesota.

Molina, R., H. Massicotes & J. M. Trappe. 1992. Specificity phenomena in mycorrhizal symbiosis: Community-ecological consequences and practical implications. Pp. 357–423 *in* M. F. Allen (editor), Mycorrhizal Functioning. Chapman & Hall, London.

Moser, M. & E. Horak. 1975. *Cortinarius* Fries und nahe verwandte Gattungen in Südamerika. Beih. Nova Hedwigia 52: 1–628.

Mueller, G. M. 1996 [1997]. Distribution and species composition of *Laccaria* (Agaricales) in tropical and subtropical America. Revista Biol. Trop. 44: (supplement 4): 131–135.

——— & R. E. Halling. 1995. Evidence for high biodiversity of Agaricales (Fungi) in neotropical montane *Quercus* forests. Pp. 303–312 *in* S. Churchill, H. Balslev, E. Forero & J. Luteyn (editors), Biodiversity and Conservation of Neotropical Montane Forests. New York Botanical Garden Press, Bronx.

——— & E. M. Pine. 1994. DNA data provide evidence on the evolutionary relationships between mushrooms and false truffles. McIlvainea 11(2): 61–74.

——— & B. A. Strack. 1992. Evidence for a mycorrhizal host shift during migration of *Laccaria trichodermophora* and other agarics into Neotropical oak forests. Mycotaxon 45: 249–256.

Newman, E. I. & P. Reddell. 1987. The distribution of mycorrhizas among families of vascular plants. New Phytol. 106: 745–751.

Pirozynski, K. A. & D. W. Malloch. 1975. The origins of land plants: A matter of mycotrophism. Biosystems 6: 153–164.

Raven, P. H. & D. I. Axelrod. 1974. Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 61: 539–673.

Read, D. J. 1993. Mycorrhiza and plant communities. Pp. 1–31 *in* I. C. Tommerup (editor), Advances in Plant Pathology—Mycorrhiza Synthesis. Academic Press, London.

Smith, S. E. & D. J. Read. 1997. Mycorrhizal Symbiosis, 2nd ed. Academic Press, San Diego.

Talent, J. A. 1984. Australian biogeography past and present: Determinants and implications. Pp. 57–93 *in* J. J. Veevers (editor), Phanerozoic Earth History of Australia. Clarendon Press, Oxford.

Thiers, H. D. 1984. The secotioid syndrome. Mycologia 76: 1–8.

Trappe, J. M. 1977a. Selection of fungi for ectomycorrhizal inoculation in nurseries. Annual Rev. Phytopathol. 15: 203–222.

———. 1977b. Biogeography of hypogeous fungi: Trees, mammals, and continental drift. 2nd Int. Mycol. Cong. Abstr. P. 675.

——— & C. Maser. 1977. Ectomycorrhizal fungi: Interactions of mushrooms and truffles with beasts and man. Pp. 165–179 *in* T. Walters (editor), Mushrooms and Man, an Interdisciplinary Approach to Mycology. USDA Forest Service, Oregon.

———, M. A. Castellano & A. W. Claridge. 2000. Continental Drift, Climate, Mycophagy, and the Biogeography of Hypogeous Fungi. Proc. 5th Congrès Int. Sci. et Culture de la truffe, Aix-en-Provence (in press).

Truswell, E. M., A. P. J. Kershaw & I. R. Sluiter. 1987. The Australian-southeast Asian connection: Evidence

from the paleobotanical record. Pp. 32–49 *in* T. C. Whitmore (editor), Biogeographical Evolution of the Malay Archipelago. Oxford Univ. Press, Oxford.

Wolfe, C. B., Jr. & N. L. Bougher. 1993. Systematics, mycogeography, and evolutionary history of *Tylopilus* subg. *Roseoscabra* in Australia elucidated by comparison with Asian and American species. Austral. Syst. Bot. 6: 187–213.

MULTIGENE PHYLOGENY OF *EPICHLOË* SPECIES, FUNGAL SYMBIONTS OF GRASSES[1]

K. D. Craven,[2] P. T. W. Hsiau,[3] A. Leuchtmann,[4] W. Hollin,[2] and C. L. Schardl[2,5]

ABSTRACT

Epichloë species are fungal symbionts (endophytes) of grasses, many of which are benign or mutualistic and have a balance of horizontal (contagious) and vertical (seed-borne) transmission, whereas others mainly transmit horizontally and are more antagonistic. Over the past eight years several *Epichloë* species have been described based largely on the biological species concept. We conducted a multi-gene phylogenetic analysis to evaluate these endophytes as phylogenetic species, and thereby assess the relationship of phylogenetic and biological species. Variation mainly in introns of genes encoding β-tubulin (*tub2*), translation elongation factor 1-α (*tef1*), and actin (*act1*) provided robust phylogenetic signal distinguishing the described *Epichloë* species. Outgroup rooting split the genus into two major groups. One group included most species with balanced transmission strategy, and in this group the phylogenetic and biological species concepts corresponded well. In contrast, these species concepts poorly corresponded for the other group, the *Epichloë typhina* complex, with predominantly antagonistic, horizontally transmitted endophytes. We suggest that the balance of vertical and horizontal transmission may promote ecological (host) specialization and subsequent genetic isolation as mechanisms promoting speciation; whereas strict horizontal transmission may select for broader host ranges, slow the development of genetically isolated species, and thereby increase lineage sorting effects that cause conflicts between phylogenetic and biological species.

Key words: biological species, Clavicipitaceae, *Epichloe*, grass endophytes, molecular phylogenetics, phylogenetic species, Poaceae, symbiosis.

Fungi in the family Clavicipitaceae (Ascomycota) are a diverse group of organisms capable of interacting with an even broader assemblage of hosts. Contained within the Clavicipitaceae are fungal and invertebrate pathogens (tribe Cordycipeae), and plant pathogens (tribes Ustilaginoideae, Clavicipeae, and Balansieae) (Diehl, 1950). Members of the tropical tribe Ustilaginoideae are biotrophs of bamboos, forming hardened sclerotic stromata on stems (Diehl, 1950), while the Clavicipeae destructively infect grass florets and replace host ovules with fungal sclerotia (Tudzynski et al., 1995). Fungi in the tribe Balansieae form perennial infections of grasses, yet cause little or no symptoms of disease. The extent of host colonization by members of this tribe varies from fairly localized, epibiotic infections to a more systemic, endophytic growth habit (Glenn et al., 1996). Species in the genus *Epichloë* lie at this endophytic extreme, systemically infecting cool-season grasses in the subfamily Pooideae (Schardl & Phillips, 1997). All *Epichloë* species are intercellular inhabitants of their host grasses, causing no visible symptoms of infection for the majority of the symbiont and host life cycles (Fig. 1). The only macroscopic sign of infection occurs as the grass reaches reproductive maturity and initiates flowering. At this stage, the endophytes may cause abortion of some or all inflorescences (grass-choke disease), and replace them with their own sexual structures (stromata). When a stroma is fertilized by spermatia from a stroma of an opposite mating type, the sexual state develops and culminates in production and ejection of contagious spores. The level of antagonism to the host is directly related to the level of fungal sexual expression, as a "strong choker" effectively shuts down host seed production (Schardl et al., 1997). While some *Epichloë* species seem to choke (stromatize) every developing host inflorescence, other species reproduce sexually on only a portion of the host tillers, the other inflorescences develop normally, and the endophyte enters into the developing ovule of the host seed. The result is vertical (asexual) transmission of the endophyte from the maternal host to her offspring.

[1] This work was supported by National Science Foundation grant DEB-9707427. This is publication number 00-12-99 of the Kentucky Agricultural Experiment Station, published with the approval of the director. The authors thank Alfred D. Byrd for expert laboratory assistance and K. O'Donnell for advice.

[2] Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0091, U.S.A.

[3] Current address: Dept. of Horticulture, Taiwan, Sugar Research Institute, No. 54 Sheng Chan Rd., Tainan, Taiwan, 701, R.O.C.

[4] Geobotanisches Institut ETH, Zollikerstrasse 107, CH-8008 Zürich, Switzerland.

[5] Corresponding author. Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0091, U.S.A.

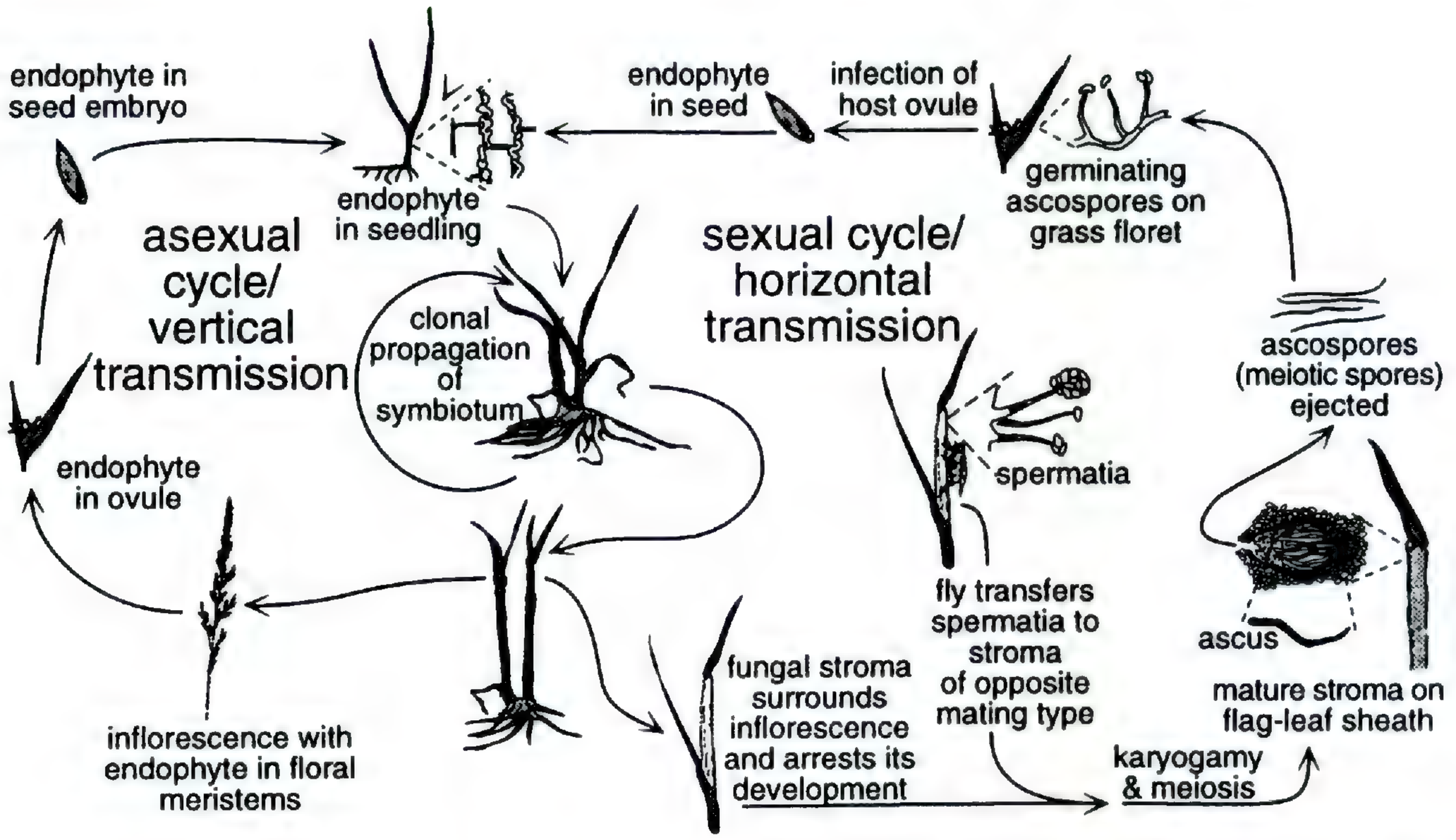


Figure 1. Generalized life cycle of *Epichloë* species using *E. festucae* in *Festuca rubra* as an example. Both transmission life cycles are exhibited by several *Epichloë* species (see text). Some *Epichloë* species (such as *E. typhina*) exhibit little or no asexual transmission in host seeds and are, therefore, predominantly transmitted horizontally via ascospores. Asexual derivatives of *Epichloë* species, conventionally classified in genus *Neotyphodium*, rely entirely on vertical transmission for their dissemination. Modified from Schardl et al. (1997).

Previous research based mainly on artificial mating tests has capitalized on the presence of the *Epichloë* sexual cycle and heterothallism (obligate outcrossing; White & Bultman, 1987) to circumscribe interfertility groups (mating populations) that correspond to, or approximate, biological species (Leuchtmann et al., 1994). Here we use, as a working definition of biological species, populations whose members have the potential to interbreed in nature to produce viable offspring, but who cannot interbreed with members of other species (Claridge et al., 1997; Mayr, 1940). To date, nine mating populations of *Epichloë* have been identified (Schardl et al., 1997).

Many grass endophytes appear to be restricted to an asexual lifestyle, and are only transmitted vertically (Sampson, 1933). In fact, the presence of such endophytes was widely recognized only after the realization that forage grasses infected with these asexual species were toxic to livestock such as cattle and sheep (Bacon et al., 1977). Subsequent studies have shown these endophytes to be responsible for producing novel secondary metabolites responsible for the toxicoses (Kennedy & Bush, 1983), as well as conferring on their hosts increased drought tolerance (Arechavaleta et al., 1989) and many other dramatic fitness enhancements (Schardl & Phillips, 1997). Despite the apparent inability of these asexual endophytes to spread contagiously, they can be abundant because they are seed transmitted at nearly 100% efficiency (Siegel et al., 1984). Although their hyphal morphology, conidiophore size and structure, and host associations all indicate their relatedness to *Epichloë* species, their lack of a sexual phase has precluded their classification based on a biological species concept.

The development of modern molecular techniques such as polymerase chain reaction (PCR) and DNA sequencing allows classification based on genetic relationships (phylogenies) to be considered. The strength of using gene sequence data for inferring relationships arises from a wealth of precisely comparable characters (aligned positions of nucleotide sequences) between even the most distantly related species (Felsenstein, 1988). Relationships emerging from such analyses have, in many cases, corroborated classification schemes based on morphological characteristics (Hillis, 1987). Molecular data have also been used to either strengthen or weaken support for hypotheses regarding organismal and cellular evolution (Hillis, 1987). For example, gene sequence data indicate that cellular organelles such as mitochondria and chloroplasts are most closely related to some free-living bacterial species, lending credence to the endosymbiont theory of the modern-day eukaryote cell as a composite of two or more once distinct prokaryote species (Margulis, 1991).

Gene sequence data also allows phylogenetic

inferences for organisms that lack distinguishing features for reliable morphological classification. Because of rather little morphological variation many sexual endophytes were originally classified as *Epichloë typhina.* Recently, a number of species have been described based on molecular phylogenetic evidence, interfertility groups, and morphology (Leuchtmann & Schardl, 1998; Leuchtmann et al., 1994; Schardl & Leuchtmann, 1999; White, 1993, 1994). A phylogeny has been inferred from partial sequences of 26S large subunit rRNA genes from *Epichloë* species and other members of the family Clavicipitaceae (Kuldau et al., 1997). Those endophytes assigned to the genus *Epichloë* through morphological data and mating tests all formed a monophyletic clade within the Clavicipitaceae. A more comprehensive analysis within the genus *Epichloë* was conducted using non-coding regions of the β-tubulin gene (*tub2*) (Schardl et al., 1997). Each sexual isolate contained a single *tub2* copy, reflecting the haploid state of the *Epichloë* mycelium. Most but not all mating populations correspond to well defined clades in phylogenetic analyses based mainly on *tub2* introns. However, more detailed analyses are needed to further test the inferred relationships.

Similar analysis of *tub2* sequence data obtained from the strictly asexual grass endophytes revealed quite novel findings. The majority of these isolates actually appeared to have multiple *tub2* copies (Schardl et al., 1994; Tsai et al., 1994; Moon et al., 2000; K.D.C. & C.L.S., unpublished obs.). Through a combination of restriction endonuclease digestion and selective priming in PCR, as well as by cloning, these copies could be isolated and sequenced independently. Inclusion of these sequences in a phylogenetic analysis of *Epichloë* species revealed that each *tub2* copy contained in the genome of an asexual grass endophyte aligned most closely with that of a different sexual *Epichloë* species (Schardl et al., 1994; Tsai et al., 1994). For example, *Neotyphodium coenophialum,* a common asexual endophyte of tall fescue [*Lolium arundinaceum* (Schreb.) S. J. Darbyshire = *Festuca arundinacea* Schreb.] was found to harbor three *tub2* copies corresponding with those of three different *Epichloë* species: *E. festucae, E. typhina,* and *E. baconii* (Tsai et al., .994). Similar results were obtained by analysis of ther genes (specifically, those utilized in this paper; K.D.C. & C.L.S., unpublished data). Furthermore, other asexual endophytes from *Festuca* L. spp., *Lolium* L. spp., *Poa* L. spp., and *Stipa* L. spp. have genes derived from different combinations of *Epichloë* species, such as *E. baconii* with *E. typhina* (Tsai et al., 1994), *E. baconii* with *E. bromicola* (Moon et al., 2000), *E. amarillans* with *E. typhina* (Schardl & Wilkinson, 2000), *E. festucae* with *E. typhina* (K.D.C., unpublished), *E. festucae* with *E. elymi, E. bromicola* with *E. typhina,* and *E. bromicola* with *E. festucae* (K.D.C., unpublished). Based on these findings, most asexual endophytes related to *Epichloë* spp. are likely to be genetic hybrids arising through the parasexual process of hyphal anastomosis (Schardl et al., 1994; Schardl & Wilkinson, 2000), and the asexual tall fescue endophyte is apparently derived from at least two such hybridizations. The genus *Neotyphodium* has been adopted to contain such endophytes with close relationships to *Epichloë* spp., but lacking a sexual stage in their life cycles (Glenn et al., 1996). A morphological difference that often distinguishes *Neotyphodium* spp. from their *Epichloë* relatives is that the asexual endophytes tend to have larger conidia, probably as a result of increased genome content due to hybrid origins (Kuldau et al., 1999).

A potential problem that arises when sequence data are used to construct phylogenies is whether or not the gene tree accurately represents the true evolutionary relationships between species (species tree). For example, similarities between two sequences can arise from convergence, gene conversion, or homology (common ancestry). Homology can further be divided into orthology (common ancestry tracing back to a speciation event) and paralogy (common ancestry tracing back to a gene duplication event) (Moritz & Hillis, 1996). Sampling of paralogous sequences can cause incorrect inferences about species phylogeny, so only orthologous sequences should be evaluated. Therefore, the genes chosen in such analyses are of paramount importance if one wishes to obtain an accurate evolutionary picture.

Another way of measuring the accuracy of a gene tree in reflecting the actual species tree is to sample other genes. If trees developed from different, independently assorting genes contain identical branching orders and taxa placement, this can be regarded as strong support for these estimations as accurately revealing the evolutionary relationships among the species being studied. An additional advantage to multi-gene phylogenetics is that populations that are, or have been, interbreeding can be distinguished from those that are genetically isolated in nature (Geiser et al., 1998; Burt et al., 1996). This is important for fungal evolutionary biology because on the one hand many lack a known sexual stage, and on the other hand the ability to mate in experimental crosses (biological species) does not guarantee that the process is relevant to natural populations. This paper reports the char-

acterization and analysis of two more genes in the *Epichloë* genome. Portions of the translation elongation factor 1-α (*tef1*) gene (O'Donnell et al., 1998) and the actin (*act1*) gene (Fidel et al., 1988), with variable introns, were amplified and sequenced for this group of closely related grass endophytes. The sequence data were used to construct separate gene phylogenies, which could be compared to those obtained from *tub2* sequences. The results indicate different phylogenetic patterns for symbiont species that themselves differ in relative mutualistic or antagonistic character.

Materials and Methods

Biological materials

Fungal isolates, their hosts, and sequence accession numbers are listed in Table 1. Endophytes were isolated by plating infected, surface-sterilized leaf sheath and stem material onto potato dextrose agar (PDA, Difco Laboratories, Detroit, Michigan) containing streptomycin and penicillin (1 mg L^{-1} each). Plates were checked regularly for contamination and endophytic growth. Most strains grew out 5–14 days after plating. Isolates were stored in 50% glycerol at −80°C (Schardl & An, 1993) and on PDA slant cultures overlain with autoclaved light mineral oil.

Mating tests

Plants naturally infected with *Epichloë* were vernalized over winter at an experimental garden in Zurich, Switzerland, to induce stromata formation. The following spring, plants were moved into a greenhouse where mating tests were performed on emergent stromata. Spermatia were transferred between mating types by rubbing stromata of opposite mating type together, or by applying cultures containing conidia onto stromata. Successful matings between opposite mating types (of the same mating population) were evidenced by the production of tan to orange perithecia after 3–6 weeks, from which ascospores could be collected. Single ascospore isolates were grown on media and subjected to isozyme analysis. Independent assortment of parental isozyme alleles in ascospore progeny confirmed successful matings.

Preparation of genomic DNA

Subcultures were generated by grinding a small amount of fungal mycelium in sterile water using an autoclaved mini-pestle and spreading approximately 300 μl of this suspension atop sterile cellophane disks on PDA plates. The permeable cellophane allowed hyphal growth and facilitated subsequent harvest of mycelium. Cultures were incubated at room temperature for two weeks, after which mycelium was harvested and freeze dried. Total fungal DNA was prepared as previously described (Byrd et al., 1990; Schardl et al., 1997) or, preferably, the method of Al Samarrai and Schmid (2000).

Assessment of gene linkage

To assess the possibility of linkage between the three genes used in this analysis, spermatia from isolate ATCC200736 (*Epichloë typhina* from *Lolium perenne*) were used to fertilize stromata of isolate ATCC200740 (*E. typhina* from *Dactylis glomerata*). Since these two *E. typhina* isolates are of opposite mating type, perithecia developed from which ascospores were obtained. Inheritance of parental alleles in each progeny was assessed by polymerase chain reaction (PCR) with primers specific for each *tub2*, *tef1*, and *act1* allele. All primers are listed in Table 2. Amplification of *tub2* spanning introns 1–3 was performed using non-selective primer tub2-exon1d–1 with selective primers tub2-intron3u–200736 (selective for *E. typhina* ATCC200736) and tub2-intron3u–200740 (selective for *E. typhina* ATCC200740). The non-selective primer annealed to the exon 1–intron 1 junction while both selective primers annealed to complementary regions within intron 3. A region of the *act1* gene approximately 300 bp long (including introns 2–4) was amplified using two selective primers for each parental isolate. Primers act1-intron2d–200736 and act1-intron-4u-200736, respectively, annealed to sites within introns 2 and 4 of the ATCC200736 *act1* allele. Similarly, primers act1-intron2d–200740 and act1-intron4u–200740 annealed to these same regions of the ATCC200740 *act1* allele. Partial amplification of the *tef1* gene, including introns 1–2, was performed using the non-specific primer tef1-exon1d with specific primers tef1-intron3u–200736 for ATCC200736 or tef1-intron3u–200740 for ATCC200740. The non-specific primer anneals to conserved sequence within exon 1, while both isolate specific primers anneal to complementary sequence within intron 4. PCR was performed in 50 μl reactions containing 0.2 μM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP; Boehringer Mannheim, Indianapolis, Indiana), 1 X PCR buffer (10 mM tris(hydroxymethyl)aminomethane hydrochloride [Tris-HCl] pH 8.3, 15 mM $MgCl_2$), 500 nM of each primer (see below), 10 ng total fungal DNA, and 0.02 U μl^{-1} of AmpliTaq gold DNA polymerase (Perkin-Elmer, Foster City, California). The thermal cycler (Perkin-Elmer model 2400) was programmed as fol-

Table 1. *Epichloë* species used in this study. All isolates are housed in permanent reference storage at the American Type Culture Collection (ATCC) except those designated with E prefixes, which are kept in cold storage at the University of Kentucky. nd = not determined.

Fungal species	Isolate	Host species	GenBank accession number		
			act1	*tub2*	*tef1*
Claviceps purpurea (Fr.) Tul.	ATCC20102	*Secale cereale* L.	AF276509	AF062646	AF276508
Echinodothis tuberiformis (B. & Rav.) Atk.	ATCC201937	*Arundinaria tecta* (Walter) Muhl.	AF276511	L78268	AF276510
Epichloë amarillans J. F. White	ATCC200743	*Sphenopholis obtusata* (Michx.) Scribn.	AF240082	L06958	AF231191
E. amarillans	ATCC201670	*Sphenopholis obtusata*	nd	AF062426	nd
E. amarillans	ATCC200744	*Agrostis hiemalis* (Walter) Britton et al.	AF240083	L06959	AF231192
E. amarillans	ATCC90815	*Agrostis hiemalis*	nd	AF250732	nd
E. baconii J. F. White	ATCC200745	*Calamagrostis villosa* (Chaix) Gmelin	AF240086	L78270	AF231196
E. baconii	ATCC76552	*Agrostis stolonifera* L.	AF240084	L06961	AF231193
E. baconii	ATCC90167	*Agrostis tenuis* Sibth.	AF240085	L78279	AF231194
E. baconii	ATCC200746	*Agrostis tenuis*	nd	AF250733	AF231195
E. brachyelytri Schardl & Leuchtmann	ATCC200752	*Brachyletrum erectum* (Schreb.) Beauv.	AF240087	L78271	AF231197
E. brachyelytri	ATCC200753	*Brachyletrum erectum*	nd	AF250734	AF231198
E. brachyelytri	ATCC200754	*Brachyletrum erectum*	nd	AF250735	AF231199
E. brachyelytri	ATCC201561	*Brachyletrum erectum*	AF240088	AF062427	AF231200
E. brachyelytri	ATCC201560	*Brachyletrum erectum*	nd	AF250736	AF231201
E. bromicola Leuchtmann & Schardl	ATCC200749	*Bromus erectus* Huds.	AF240089	L78289	AF231202
E. bromicola	ATCC200750	*Bromus erectus*	AF240090	L78290	AF231203
E. bromicola	ATCC201558	*Bromus ramosus* Huds.	AF240091	AF062430	AF231204
E. bromicola	ATCC201559	*Bromus benekenii* (Lange) Trimen	AF240092	AF250737	AF231205
E. clarkii J. F. White	ATCC200742	*Holcus lanatus* L.	AF240093	L78281	AF231206
E. clarkii	ATCC200741	*Holcus lanatus*	nd	AF250738	AF231207
E. clarkii	E1155	*Holcus lanatus*	nd	AF250739	nd
E. clarkii	E426	*Holcus lanatus*	nd	AF250740	nd
E. clarkii	ATCC90168	*Holcus lanatus*	nd	AF250741	nd
E. elymi Schardl & Leuchtmann	ATCC200850	*Elymus virginicus* L.	AF240094	L78273	AF231208
E. elymi	ATCC201551	*Elymus canadensis* L.	AF240095	L06962	AF231209
E. elymi	ATCC201553	*Elymus virginicus*	nd	AF062428	nd
E. elymi	ATCC201554	*Elymus virginicus*	AF240096	AF250742	nd
E. elymi	ATCC201555	*Elymus villosus* Muhl. ex Willd.	nd	AF250743	nd
E. elymi	ATCC201556	*Elymus hystrix* L.	AF240096	AF250744	nd
E. elymi	ATCC201557	*Elymus hystrix*	nd	AF250745	nd
E. festucae Leuchtmann, Schardl, & Siegel	ATCC90661	*Festuca rubra* subsp. *rubra* (Gaud.) Hayek	AF240098	L06955	AF231210
E. festucae	ATCC201550	*Festuca rubra* subsp. *commutata* Gaudin	AF240100	L06957	AF231211

Table 1. Continued.

Fungal species	Isolate	Host species	GenBank accession number		
			act1	*tub2*	*tef1*
E. festucae	ATCC90660	*Festuca rubra* subsp. *commutata*	nd	AF250746	AF231214
E. festucae	E028	*Festuca longifolia* Thuill.	AF240099	L06956	AF231213
E. festucae	E434	*Lolium giganteum* (L.) S. J. Darbyshire	nd	L78286	AF231212
E. cf. *festucae*	E1157	*Koeleria cristata* Pers.	nd	AF250747	AF231215
E. glyceriae Schardl & Leuchtmann	ATCC200747	*Glyceria striata* (Lam.) Hitchc.	AF240101	L78275	AF231216
E. glyceriae	ATCC200755	*Glyceria striata*	AF240102	L78276	AF231217
E. sylvatica Leuchtmann & Schardl	ATCC200748	*Brachypodium sylvaticum* (Huds.) P. B.	AF240104	L78278	AF231218
E. sylvatica	ATCC200751	*Brachypodium sylvaticum*	AF240103	L78291	AF231219
E. typhina (Pers.:Fr.) Tul.	ATCC200736	*Lolium perenne* L.	AF240105	X52616	AF231220
E. typhina	E432	*Lolium perenne*	AF240106	AF250752	AF231221
E. typhina	ATCC200738	*Anthoxanthum odoratum* L.	AF240107	L78288	AF231222
E. typhina	ATCC200737	*Anthoxanthum odoratum*	nd	AF250752	nd
E. typhina	ATCC200739	*Brachypodium pinnatum* (L.) P. Beauv.	AF240108	L78292	AF231223
E. typhina	E1016	*Brachypodium pinnatum*	AF240109	AF250749	AF231224
E. typhina	ATCC200740	*Dactylis glomerata* L.	AF240110	L78274	AF231225
E. typhina	ATCC200849	*Dactylis glomerata*	nd	L78287	nd
E. typhina	ATCC200851	*Phleum pratense* L.	AF240111	L78280	AF231226
E. typhina	E348	*Phleum pratense*	AF240112	L78277	AF231227
E. typhina	ATCC201667	*Poa nemoralis* L.	AF240114	AF062429	AF231229
E. typhina	ATCC201668	*Poa nemoralis*	AF240115	AF250756	AF231230
E. typhina	E1015	*Poa trivialis* L.	nd	AF250748	nd
E. typhina	E1153	*Poa trivialis*	nd	AF250751	nd
E. typhina	E428	*Poa trivialis*	nd	AF250755	nd
E. typhina	E1158	*Arrhenatherum elatius* (L.) P. Beauv.	nd	AF250753	nd
E. typhina	ATCC201666	*Poa silvicola* Guss.	AF240113	L78285	AF231228
E. typhina	E1019	*Poa silvicola*	nd	AF250750	nd
E. typhina	ATCC201669	*Poa pratensis* L.	AF240116	L78284	AF231231
E. typhina	E1154	*Poa pratensis*	nd	AF250757	nd
E. typhina	E1020	*Poa pratensis* L. subsp. *angustifolia* (L.) Lej.	nd	nd	AF231232

Table 2. Oligonucleotide primers used in this study. Primers are listed in order of appearance in text.

Oligonucleotide designation	Gene	Sequence (5′–3′)	Position	Orientation
tub2-exon1d-1	*tub2*	GAGAAAATGCGTGAGATTGT	exon 1–intron 1 junction	downstream
tub2-intron3u-200736	*tub2*	GCATGATAACACTTTTCCTTTCCA	intron 3	upstream
tub2-intron3u-200740	*tub2*	GCATGATAACACGTTTTATTTGCT	intron 3	upstream
act1-intron2d-200736	*act1*	GCGTAGACCCACGCAACGTA	intron 2	downstream
act1-intron4u-200736	*act1*	GTGCATGTTTGCCAGCCAGA	intron 4	upstream
act1-intron2d-200740	*act1*	GCATAGACCCACGCAACGTG	intron 2	downstream
act1-intron4u-200740	*act1*	GTGCATGTTTGCCAGCCAGT	intron 4	upstream
tef1-exon1d	*tef1*	GGGTAAGGACGAAAAGACTCA	exon 1	downstream
tef1-intron3u-200736	*tef1*	ATCACGCGAATGTCATGTTG	intron 4	upstream
tef1-intron3u-200740	*tef1*	ATCACGCGAATGTCATGTTA	intron 4	upstream
tub2-exon4u-1	*tub2*	TGGTCAACCAGCTCAGCACC	exon 4	upstream
tef1-exon6u-1	*tef1*	CGGCAGCGATAATCAGGATAG	exon 6	upstream
act1-exon1d-1	*act1*	TAATCAGTCACATGGAGGGT	exon1–intron 1 junction	downstream
act1-exon6u-1	*act1*	AACCACCGATCCAGACAGAGT	exon 6	upstream
act1-exon3u-1	*act1*	CTTGCACATGCCCGAACTACC	exon 3	upstream
act1-exon3d-1	*act1*	GGTAGTTCGGGCATGTGCAAG	exon 3	downstream

lows: one incubation at 94°C for 9 min.; then 40 cycles of 94°C for 60 sec., 60°C for 60 sec., and 72°C for 60 sec.

SEQUENCING OF GENOMIC DNA SEGMENTS

Sequences of each gene were obtained directly from PCR-amplified products. All oligonucleotide primers used for PCR and sequencing are listed in Table 2 along with their corresponding positions and orientations in the genes. The 5′ portions of *tub2*, inclusive of introns IVS1, IVS2, and IVS3, were amplified using non-selective primers tub2-exon1d–1 and tub2-exon4u–1. A ca. 850 bp fragment of *tef1*, including the first five introns, was amplified using primers tef1-exon1d–1 and tef1-exon6u–1. A ca. 700 bp fragment of the *act1* gene, including introns 1–5, was amplified by PCR using primers act1-exon1d–1 and act1-exon6u–1. Internal regions of the PCR-amplified product were sequenced with primers act1-exon3u–1 and act1-exon3d–1. All amplification products were verified by agarose gel (0.8%) electrophoresis. Water blanks were included as controls to identify possible contamination. Successfully amplified products were purified for sequencing using Quiaquick spin columns (Quiagen, Chatsworth, California). PCR was performed as described above, except that the temperature program was as follows: one incubation at 94°C for 9 min.; then 40 cycles of 94°C for 60 sec., 55°C for 60 sec., and 72°C for 60 sec. Reaction mixtures were as described above.

PCR products were sequenced by the Sanger method (Thomas & Kocher, 1993) with a Perkin-Elmer GeneAmp PCR System 2400 using rhodamine-labeled dideoxynucleotide triphosphates or BigDye Terminator Cycle sequencing kit (Perkin-Elmer). Sequence reaction products were analyzed using an ABI model 310 capillary electrophoresis genetic analyzer (Perkin-Elmer). Both DNA strands were sequenced.

PHYLOGENETIC ANALYSIS

Sequences were aligned using the PILEUP program in the Wisconsin Package (GCG, 1996). PILEUP parameters were adjusted empirically; a gap penalty of one and a gap extension penalty of zero resulted in accurate alignment of intron-exon junctions and in a reasonable alignment of the introns within the ingroup (*Epichloë/Neotyphodium* spp.) for individual analysis of *tub2*, *act1*, and *tef1* genes, and for a combined data set using appended sequences of all three genes for representative isolates from each *Epichloë* species, as well as from outgroup taxa, *Claviceps purpurea* ATCC20102 and *Echinodothis tuberiformis* ATCC201937. Both outgroup taxa are in the family Clavicipitaceae and are among the closest known relatives of *Epichloë* (Glenn et al., 1996). No ambiguous alignments were evident for the ingroups, but slight realignment of outgroup sequences were needed and were performed by eye. Maximum parsimony (MP) employed the branch-and-bound option in PAUP* version 4.0 beta 4 (Swofford, 1998) for exact solutions. For parsimony analysis, character changes were unweighted and unordered; gaps were treated as missing information. Robustness of the data was estimated by bootstrap replications (Swofford et al., 1996). All branches receiving 70% or higher boot-

Table 3. Linkage analysis between *act1*, *tub2*, and *tef1-alpha* genes. Parental alleles represented by letters a (for *E. typhina* ATCC200736) and b (for *E. typhina* ATCC200740). Parental isolates were chosen based on differential polymorphism in each allele detectable by selective primers in PCR.

Fungal isolate	*act1*	*tef1*	*tub2*	*act1/tef1*	*act1/tub2*	*tef1/tub2*
E. typhina parental isolates used in cross						
ATCC200736	a	a	a	aa	aa	aa
ATCC200740	b	b	b	bb	bb	bb
Progeny isolates obtained						
E386.003	a	a	b	aa	ab	ab
E386.009	a	b	a	ab	aa	ba
E386.026	b	b	a	bb	ba	ba
E386.027	b	b	b	bb	bb	bb
E386.042	a	b	b	ab	ab	bb
E386.043	a	b	b	ab	ab	bb
E386.045	a	a	a	aa	aa	aa
E386.057	a	b	b	ab	ab	bb
E386.084	a	b	a	ab	aa	ba
E386.180	a	b	a	ab	aa	ba
aa : bb : ab : ba				2:2:6:0	4:1:4:1	1:4:1:4

strap values were considered well supported. For distance-based analysis a number of different methods were employed to derive pairwise distances. These included Jukes-Cantor and Kimura two-parameter distances [transition/transversion (ts/tv) = 2], and various gamma values to adjust for between-site variation. Distance trees were inferred by neighbor-joining (NJ) implemented in PAUP* version 4.0 beta 4 (Swofford, 1998) with random taxon additions. All such distance trees were similar to the MP trees, and are therefore not shown. Maximum likelihood (ML) trees were constructed using PUZZLE in PAUP* version 4.0 beta 4. A Hasegawa-Kishino-Yano model (Swofford et al., 1996) was employed with transition/transversion = 2.0. PUZZLE uses quartet puzzling to create trees for all possible quartets of taxa via maximum likelihood. These quartet trees serve as starting points to reconstruct a set of optimal trees for all taxa. The majority-rule consensus of these trees defines the quartet puzzling tree and shows well-supported branches (Strimmer & von Haeseler, 1996). Partition homogeneity analysis was performed in PAUP* version 4.0 beta 4 on each two-gene combination in the combined sequence alignment.

Results

Linkage analysis

To check if the three genes selected for phylogenetic analysis—namely, *tub2*, *act1*, and *tef1*—were unlinked, Mendelian segregation was assessed for variant alleles in an *Epichloë typhina* cross. Ten progeny were analyzed for alleles derived from the parents, ATCC200736 (male) and ATCC 200740 (female). The occurrence of recombinant progeny was assessed for each gene pair. Ratios of parental to recombinant progeny genotypes indicated no close linkage between any of these genes in *E. typhina* (Table 3).

Mating tests

Past mating tests indicated that the currently described *Epichloë* species mostly correspond to interfertile groups (Leuchtmann & Schardl, 1998; Schardl & Leuchtmann, 1999). Exceptions were some instances of nonreciprocal interfertility among *E. typhina* isolates, and high fertility between *E. clarkii* and *E. typhina*. Several additional mating tests were conducted to establish species affinities of new isolates and mating tests that had not previously been conducted for technical reasons. A new *Epichloë* isolate from *Arrhenatherum elatius* was fully compatible in matings with *E. typhina* stromata on *Dactylis glomerata*, as were the reciprocal matings. The ascospore progeny were viable and exhibited segregation of parental allozymes. An isolate from *Poa nemoralis* was compatible with at least some strains from *Brachypodium sylvaticum*, but the matings exhibited low fertility with few asci containing ascospores and a very low germination rate of the ascospores. These matings were also confirmed by allozyme analysis. *Epichloë typhina* stromata from *P. nemoralis* mated successfully with *Brachypodium pinnatum* isolates, although fertility

was intermediate (moderate ascospore germination rate). *Epichloë clarkii* isolates from *Holcus lanatus* successfully mated with *E. typhina* stromata on *Poa trivialis*. *Epichloë festucae* fertilized with a new isolate from *Koeleria cristata* reacted by forming protoperithecia, but no mature perithecia were observed and no ascospores were obtained. *Epichloë festucae* stromata on *Festuca rubra* mated successfully with endophyte isolates from *Lolium giganteum* (= *Festuca gigantea*), *Festuca valesiaca*, *F. glauca*, and *F. heterophylla*. Matings were attempted between stromata of *E. amarillans* isolates on *Sphenopholis obtusata* and spermatia of the *E. amarillans* ex-type (ATCC200744) from *Agrostis hiemalis*. Abundant perithecia gave rise to viable ascospores, but in the 10 ascospore isolates analyzed, only maternal allozyme alleles were observed.

PHYLOGENETIC RELATIONSHIPS BASED ON *ACT1/TUB2/TEF1* COMBINED DATA

An MP tree (Fig. 2) was constructed from the combined data set of *act1*, *tub2*, and *tef1* intron sequences, and included representatives of all described *Epichloë* species and two clavicipitaceous outgroups, *Claviceps purpurea* and *Ech. tuberiformis*. The tree shown in Figure 2 reflects the overall diversity within clades and described *Epichloë* species. However, results described later indicated some incongruences between gene trees due to reticulation involving *E. typhina*. For this reason the positions of *E. typhina* isolates did not reflect their positions in all gene trees. To test that the combined analysis gave a valid result for relationships between species, it was rerun with only two representatives of *E. typhina*, and again without any *E. typhina* sequences (data not shown). In all cases the interrelationships of the remaining taxa were the same.

In bootstrap analysis (again using MP) of the combined data set most branches were strongly supported, but the placement of the root received only weak support (Fig. 3). This was not surprising because of the considerable sequence divergence between the ingroup and outgroup taxa, making unambiguous alignment difficult. Nevertheless, the inferred root was very close to the midpoint root of each individual gene tree (discussed later and shown in Figs. 4, 7, 11). Assuming its correct placement in the combined data analysis, the root split the *Epichloë* genus into two major groups (Fig. 3): One group, comprising *Epichloë amarillans*, *E. baconii*, *E. festucae*, *E. bromicola*, *E. elymi*, *E. glyceriae*, and *E. brachyelytri*, is henceforth designated

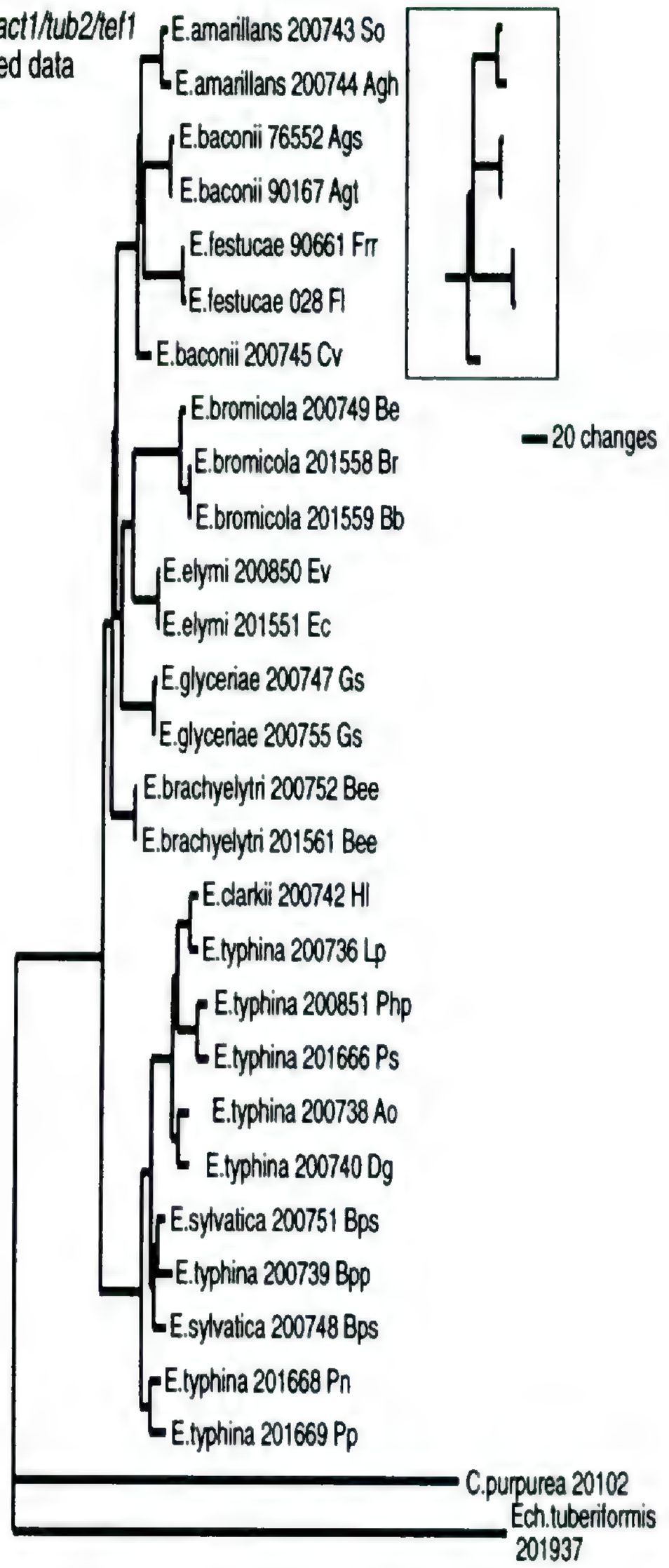


Figure 2. MP tree generated from the combined *act1/tub2/tef1* data set including representatives of each major *Epichloë* clade, *Claviceps purpurea* and *Echinodothis tuberiformis* (outgroups). Branch-and-bound search resulted in 2 MP trees, differing slightly in the *E. festucae/E. baconii/E. amarillans* clade. The alternative resolution of this clade is shown in the smaller box at right. The number of parsimony-informative characters = 379; tree length = 1350 steps; consistency index = 0.8622; retention index = 0.8502; rescaled consistency index = 0.7331; outgroup rooting with basal polytomy. Bar represents 20 inferred nucleotide substitutions. Letters following isolate number refer to abbreviated host designation. Full host names are listed in Table 1.

the "main group" of *Epichloë* species. The second group, henceforth called the "*E. typhina* complex," included *E. typhina* isolates from several host species, as well as *E. clarkii* (from *Holcus lanatus*) and *E. sylvatica* (from *Brachypodium sylvaticum*). A very strongly supported clade within the *E. typhina*

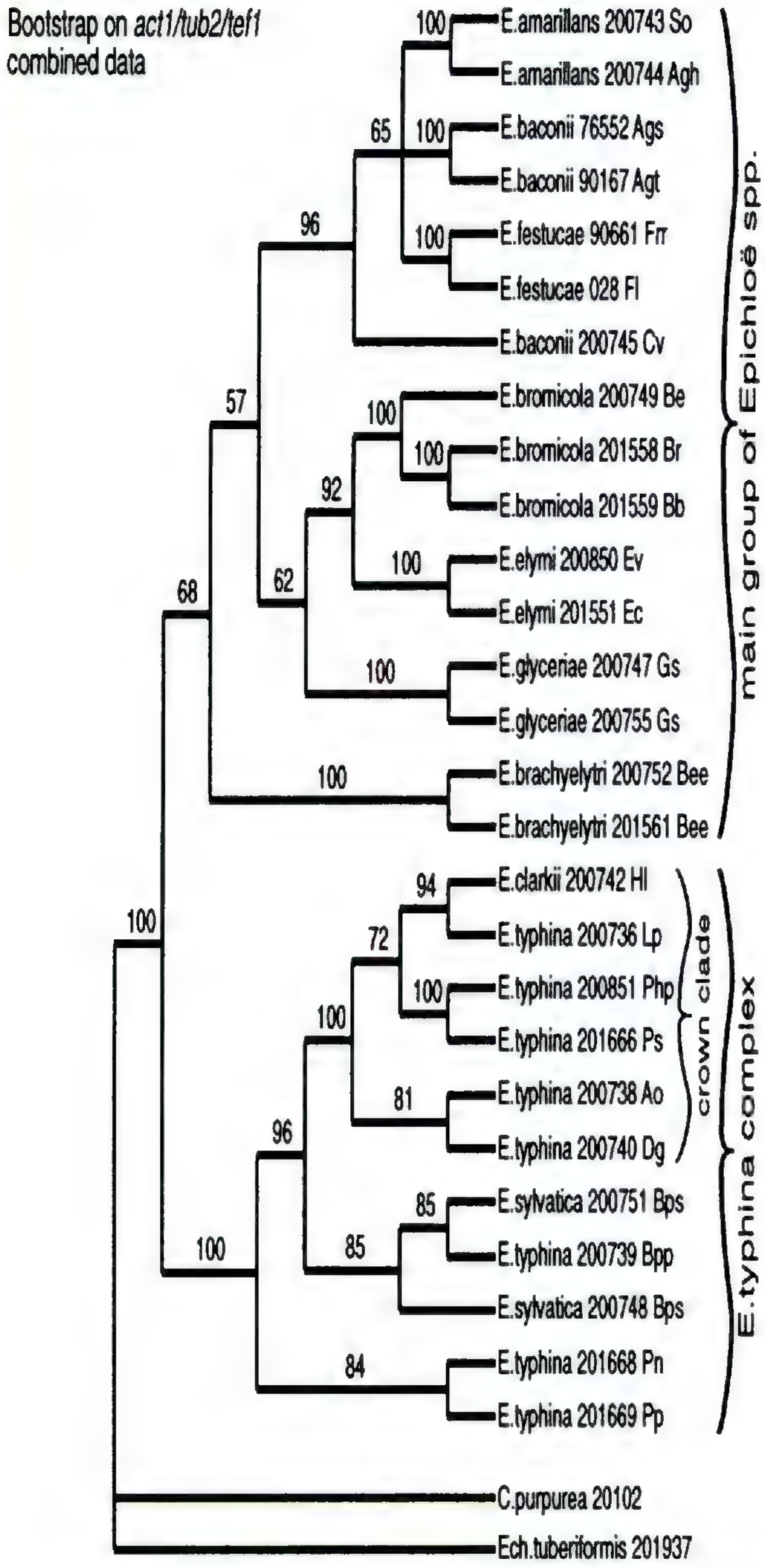


Figure 3. Bootstrap tree generated from the combined *act1/tub2/tef1* data set. Numbers at branches are the percentage of trees containing the corresponding clade based on 1000 bootstrap replications. Only bootstrap values greater than 70% are shown and considered supportive of the clades indicated by the branches.

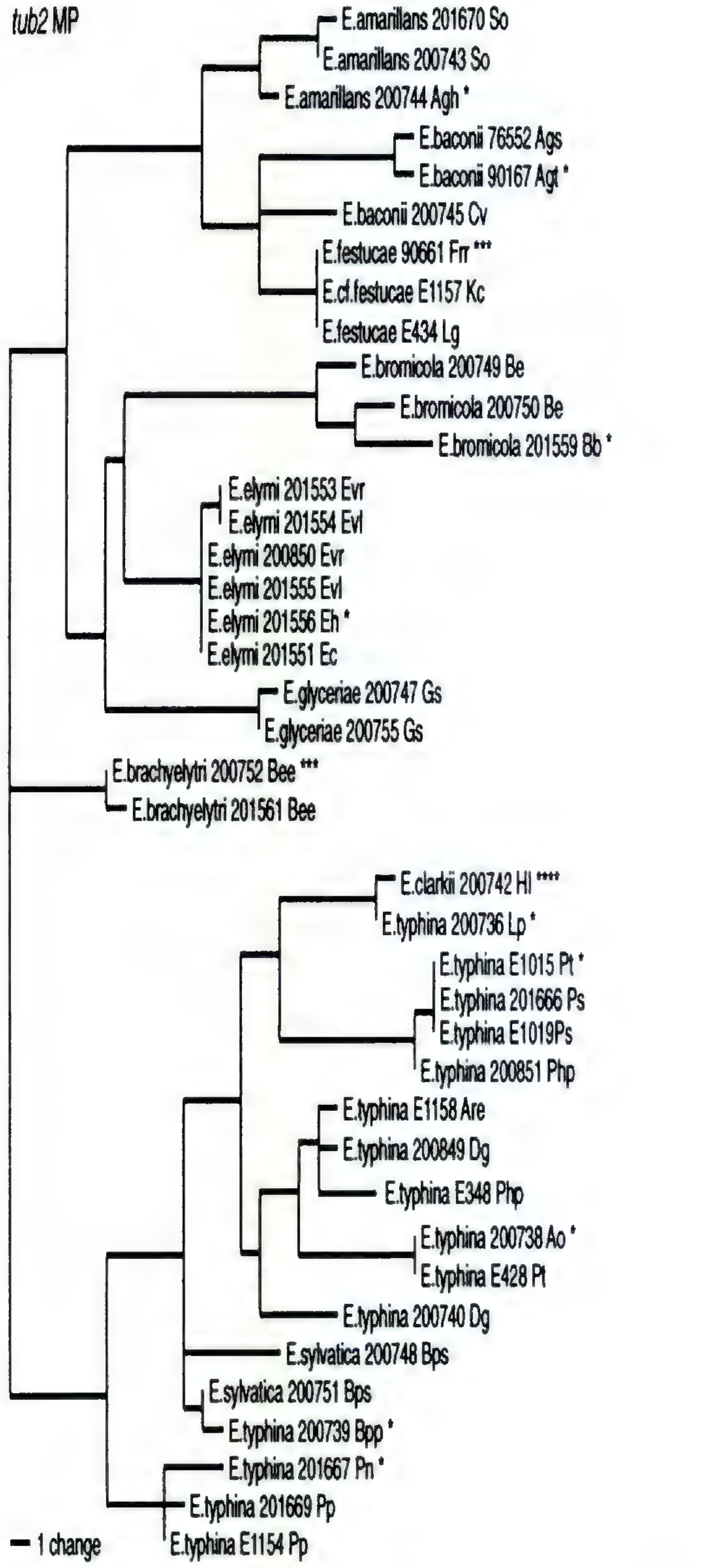


Figure 4. *Epichloë* spp. *tub2* gene tree based on maximum parsimony (MP) analysis of introns 1–3. Shown is the single MP tree obtained by branch-and-bound search. Number of parsimony-informative characters = 82; uninformative characters = 21; tree length = 143 steps; consistency index = 0.8182; retention index = 0.9426; rescaled consistency index = 0.7712; midpoint root is at the left edge. Bar represents one inferred nucleotide substitution. Asterisks (*) indicate the number of additional isolates (ranging from one to four), sampled from the same host genus, that had identical sequence.

complex was designated the crown clade (Fig. 3), and includes among others, *E. typhina* from *Dactylis glomerata* (which was among the original hosts on which Persoon described *E. typhina* and which was later selected as host of the lectotype) and *E. clarkii* from *Holcus lanatus*.

β-TUBULIN GENE PHYLOGENY

The *tub2* data set included sequences from 59 isolates of *Epichloë* spp. Phylogenies derived by MP (Figs. 4, 5), NJ (not shown), and ML quartet puzzling (Fig. 6) were consistent with each other and with the *tub2* relationships previously reported among *Epichloë* species (Schardl & Leuchtmann, 1999). Both MP bootstrap analysis (Fig. 5) and ML quartet puzzling (Fig. 6) strongly supported the two major groups elucidated by the combined data set.

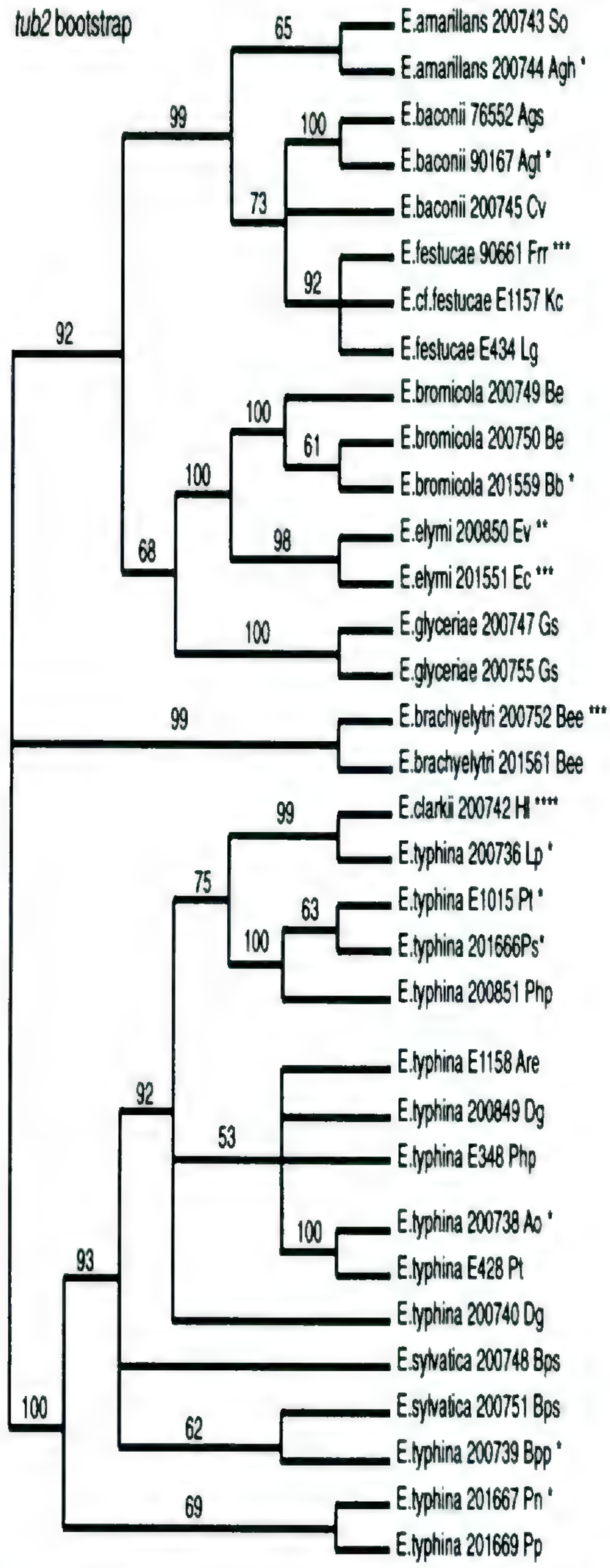


Figure 5. Bootstrap tree generated from *Epichloë tub2* gene sequence using MP (heuristic). Numbers at branches are the percentage of trees containing the corresponding clade based on 1000 bootstrap replications. Bootstrap values greater than 70% are considered supportive of the clades indicated by the branches. For bootstrap analysis each set of identical sequences was represented twice. Asterisks (*) indicate the number of additional isolates (ranging from one to four), sampled from the same host genus, that had identical sequence.

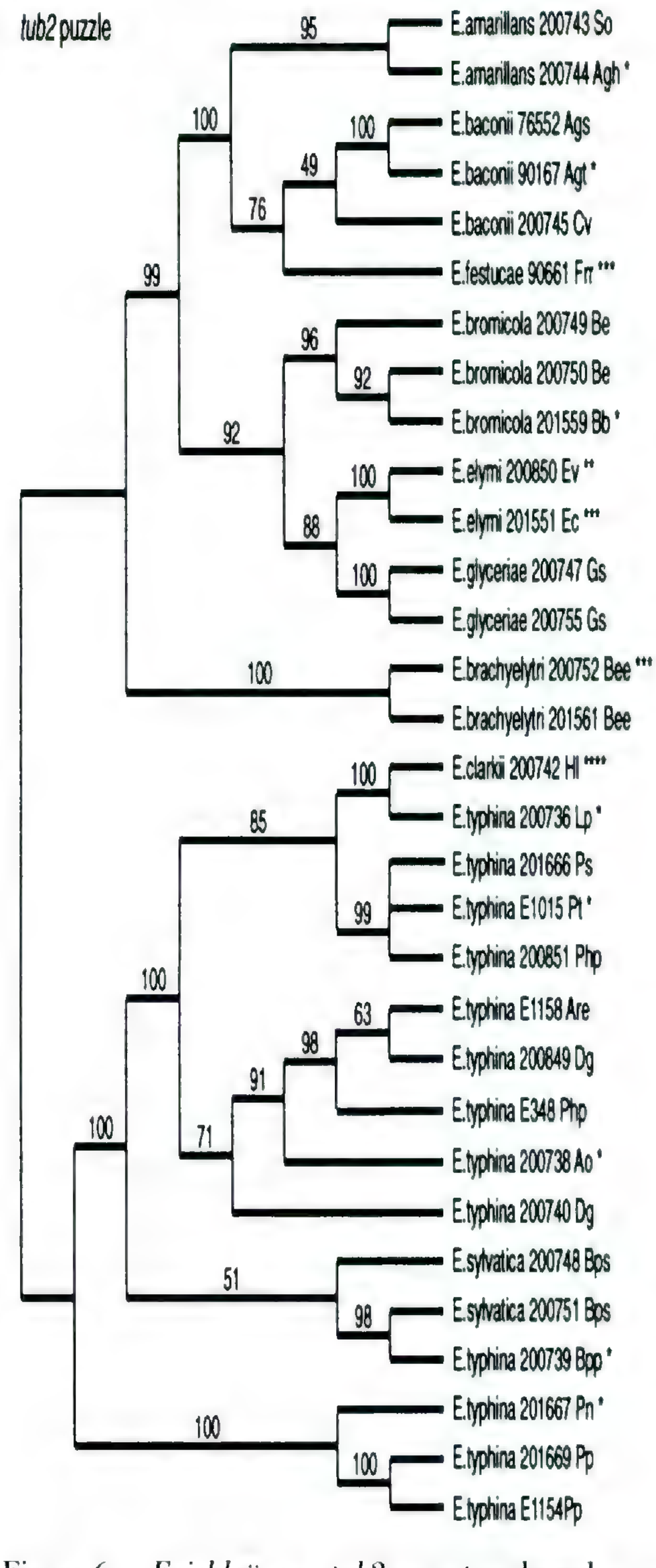


Figure 6. *Epichloë* spp. *tub2* gene tree based on maximum likelihood (ML) PUZZLE analysis of introns 1–3. Tree is based on a Hasegawa-Kishino-Yano model with ts/tv = 2 (kappa = 4.01068); number of puzzling steps = 1000. Tree shown represents majority-rule consensus and numbers at branches are estimations of support for each branch.

Members of most biological species tended to group together. For example, the isolates of *E. amarillans*, *E. brachyelytri*, *E. festucae*, *E. bromicola*, *E. clarkii*, *E. elymi*, and *E. glyceriae* grouped into exclusive clades. However, *E. baconii* from *Agrostis* spp. were not definitively grouped with *E. baconii* from *Calamagrostis villosa*, the two *E. sylvatica* isolates did not definitively group in a monophyletic clade, and *E. typhina* exhibited apparent paraphyly with *E. sylvatica* and *E. clarkii*.

The *tub2* tree included sequences from several new isolates. An isolate from host species *Koeleria cristata* was placed in the *Epichloë festucae* clade, with perfect sequence identity to four other *E. festucae* isolates. New isolates from *Arrhenatherum elatius*, *Poa silvicola*, and *P. trivialis* grouped together with isolates from several other hosts in the crown clade of the *E. typhina* complex. New isolates from *Poa pratensis* and *Brachypodium pin-*

natum grouped with other isolates from these same hosts, in basal clades of the *E. typhina* complex.

TRANSLATION ELONGATION FACTOR 1-α GENE PHYLOGENY

To assess the extent to which the *tub2* gene tree reflected the evolutionary relationships among *Epichloë* species and isolates, noncoding portions of two additional genes, *tef1* and *act1*, were sequenced from most of the isolates. A portion of *tef1* containing introns 1–4 was amplified and sequenced from representatives of the *Epichloë* species, and phylogenetic trees were inferred by MP (Figs. 7, 8), NJ (not shown), and ML quartet puzzling (Fig. 10). The *tef1* MP trees resolved most of the same clades that were resolved in the *tub2* trees, except that the positions of *E. glyceriae* and *E. brachyelytri* were unresolved in the *tef1* trees. Within the *E. typhina* complex *tub2* and *tef1* phylogenies differed in the crown clade, as would be expected if clade members are interbreeding. In bootstrap analysis, all major clades that were strongly supported by the *tub2* data were also strongly supported by *tef1* (Fig. 8). The *tef1* trees grouped isolates of each of the species, *E. brachyelytri, E. bromicola, E. clarkii, E. elymi, E. festucae,* and *E. glyceriae.* Furthermore, *E. typhina* isolates from *Lolium perenne, Poa nemoralis,* and *Brachypodium pinnatum* grouped into exclusive clades, each with strong bootstrap support.

ACTIN GENE PHYLOGENY

The *act1* MP trees were not as highly resolved as the other gene trees. This lack of resolution resulted in failure to successfully complete bootstrap analysis on the *act1* data set. Even so, all major clades indicated by *tub2* and *tef1* analyses were supported in the *act1* consensus trees (Fig. 11). One conspicuous incongruence between the *act1* phylogeny and those constructed from both *tub2* and *tef1* was the placement of the isolates within the basal clades of the *Epichloë typhina* complex. Both *tub2* and *tef1* analysis placed the *E. typhina* isolates from *Poa nemoralis* and *P. pratensis* together in the most basal clade, whereas the *act1* trees placed the *E. typhina* isolates from *Brachypodium pinnatum* together with one of the *E. sylvatica* isolates (ATCC 200751) from *Bp. sylvaticum* as the most basal clade (Figs. 11, 12). Such incongruences between gene trees in placement of taxa suggested possible genetic reticulation due to interbreeding within the *E. typhina* complex.

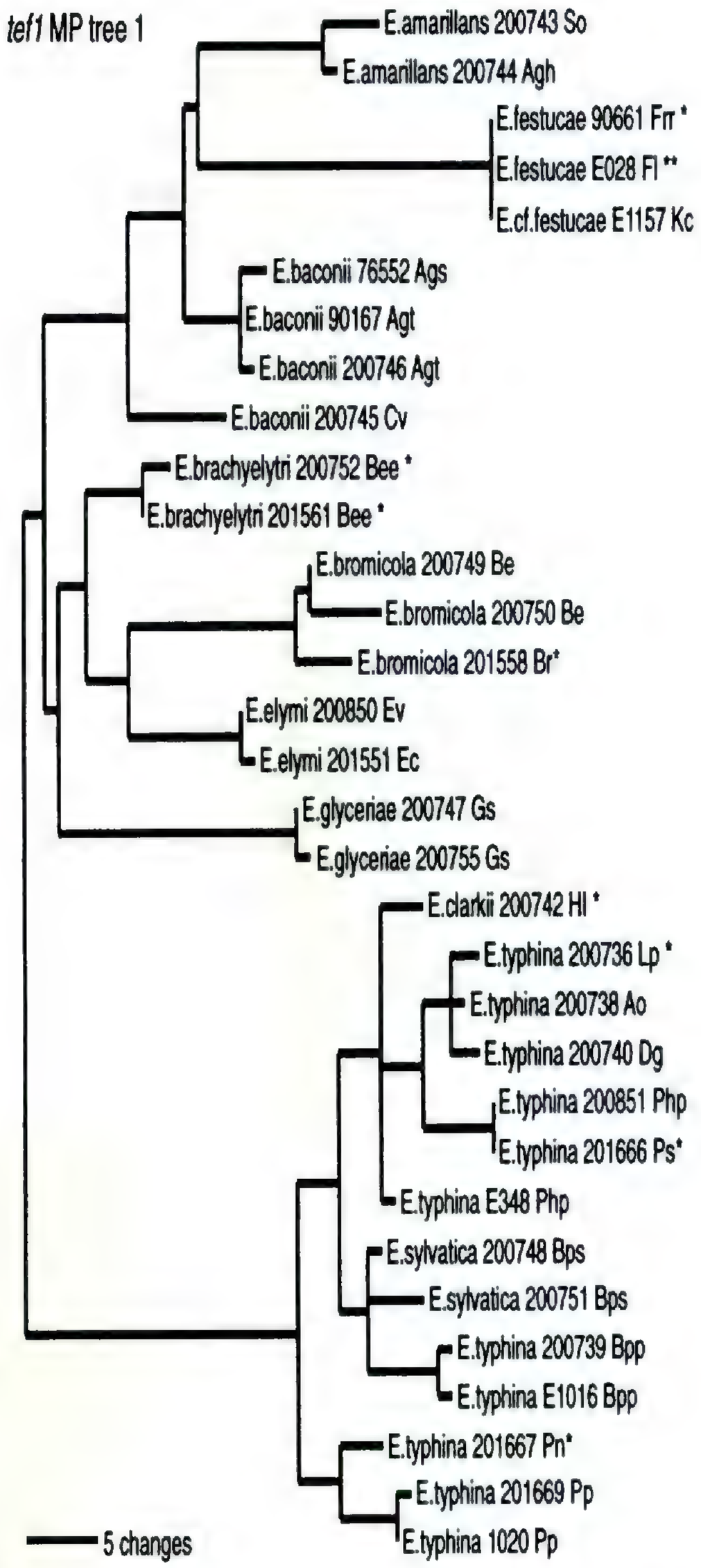


Figure 7. *Epichloë* spp. *tef1* gene tree based on MP analysis of introns 1–4. Branch-and-bound search resulted in 72 MP trees. Number of parsimony-informative characters = 110; tree length = 196 steps; consistency index = 0.8571; retention index = 0.9506; rescaled consistency index = 0.8148; midpoint root is at the left edge. Bar represents five inferred nucleotide substitutions. Asterisks (*) indicate the number of additional isolates (ranging from one to two), sampled from the same host genus, that had identical sequence. Some sequences separated on zero-length branches differ only in indels, which were treated as missing information in this analysis.

TESTS FOR TREE INCONGRUENCES

To circumscribe interbreeding populations we investigated reticulation within clades. Interbreeding should lead to incongruences between gene trees in the placement of isolates within the interbreeding population. Most clades corresponding to interfertile groups had too little sequence variation to

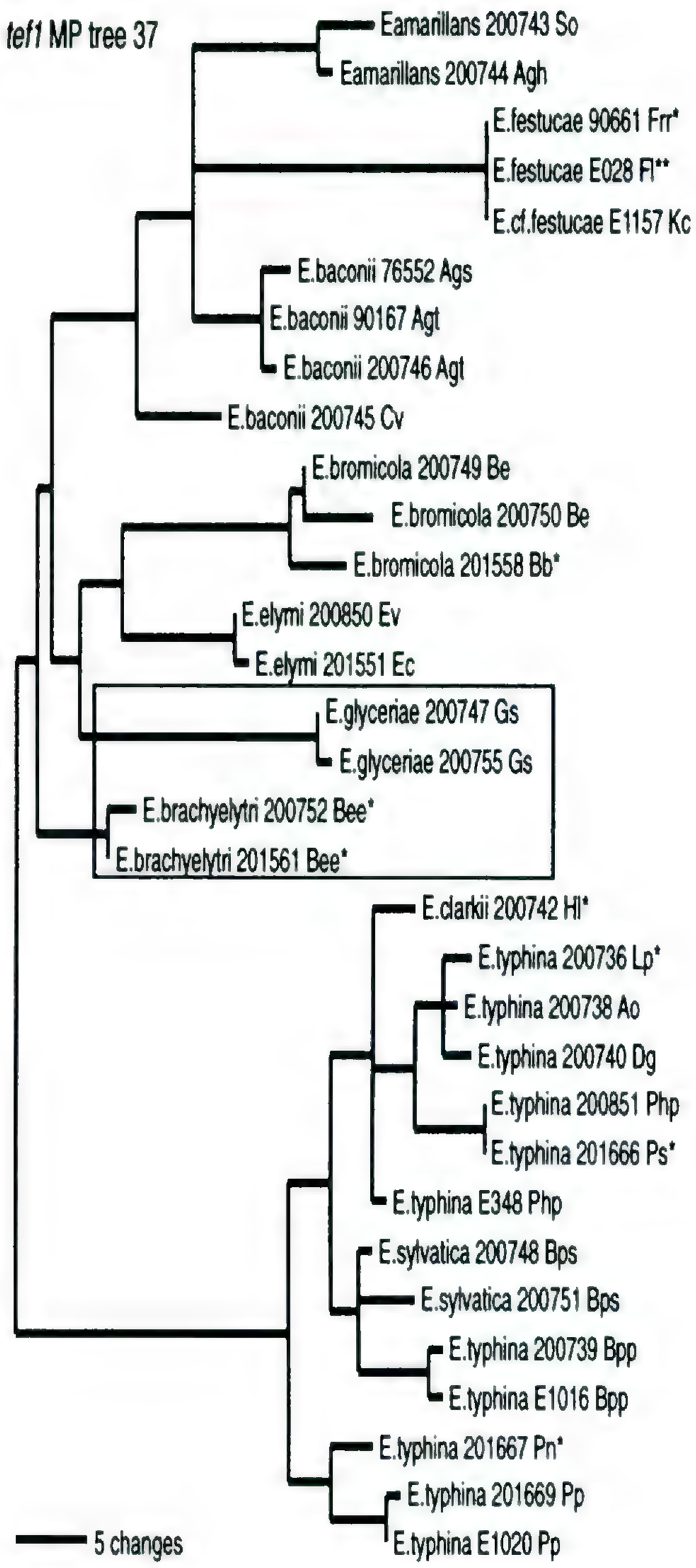


Figure 8. *Epichloë* spp. *tef1* gene tree based on MP analysis of introns 1–4. Branch-and-bound search resulted in 72 MP trees. Tree shown was chosen to represent variation in branching orders among the MP trees. The most important differences are the positions of *E. brachyelytri* and *E. glyceriae* (boxed). Number of parsimony-informative characters = 110; tree length = 196 steps; consistency index = 0.8571; retention index = 0.9506; rescaled consistency index = 0.8148; midpoint root is at the left edge. Bar represents five inferred nucleotide substitutions. Asterisks (*) indicate the number of additional isolates (ranging from one to two), sampled from the same host genus, that had identical sequence. Some sequences separated on zero-length branches differ only in indels, which were treated as missing information in this analysis.

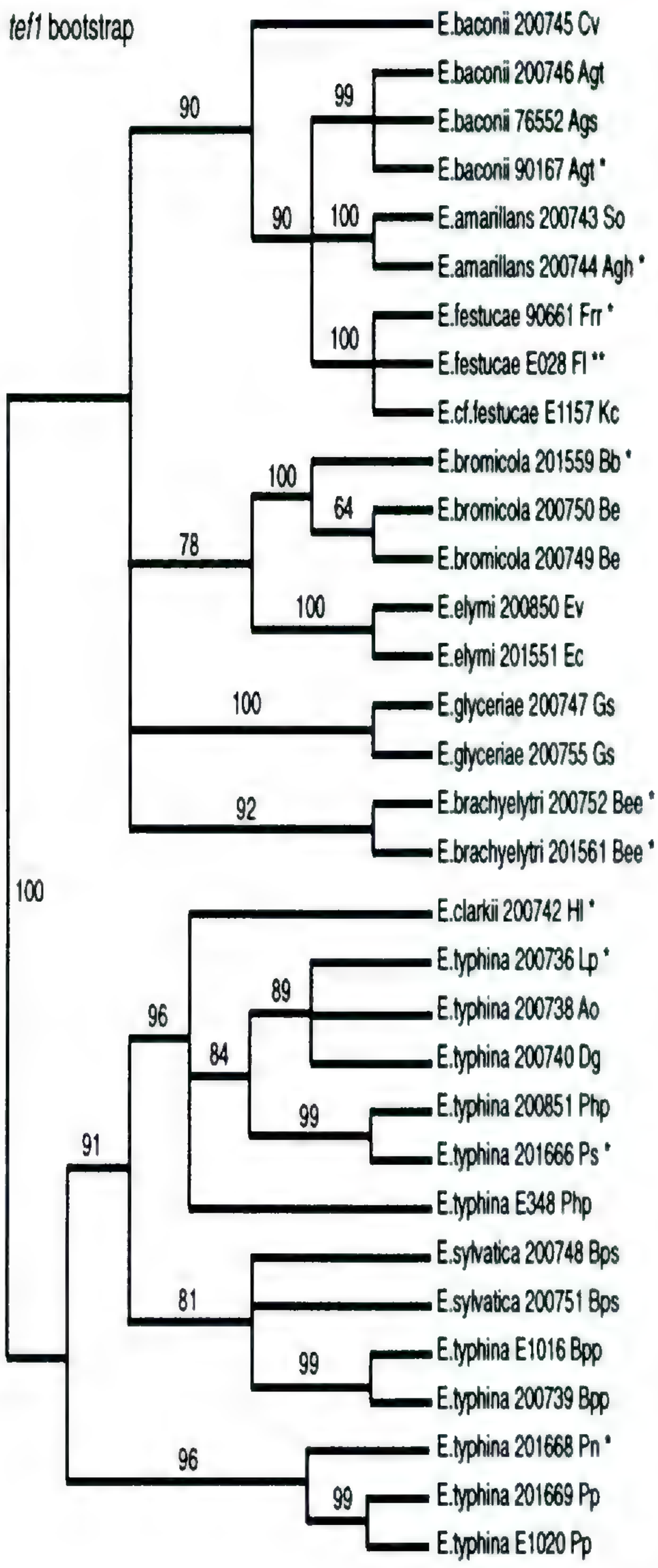


Figure 9. Strict consensus cladogram from the 72 MP trees derived from a branch-and-bound search of *tef1* sequence. Numbers at branches are the percentage of trees containing the corresponding clade based on 500 bootstrap replications. Bootstrap values greater than 70% are considered supportive of the clades indicated by the branches. For bootstrap analysis each set of identical sequences was represented only once.

make such an assessment. However, three such groups were highly diverse in sequences: *Epichloë baconii*, *E. sylvatica*, and the *E. typhina/E. clarkii* group. In all three gene trees (Figs. 4, 7, 11) the *E. baconii* isolate from *Calamagrostis villosa* was well separated from those with *Agrostis* spp. hosts. Thus, there was no conflict between the gene trees that would suggest intermating between *E. baconii* in different clades. There were, however, incongruences between gene trees in the *E. typhina* complex. Specifically, there were differences in the crown clade between all gene trees, and there were differences in basal clades of the complex when the *act1* tree was compared to the *tub2* and *tef1* trees.

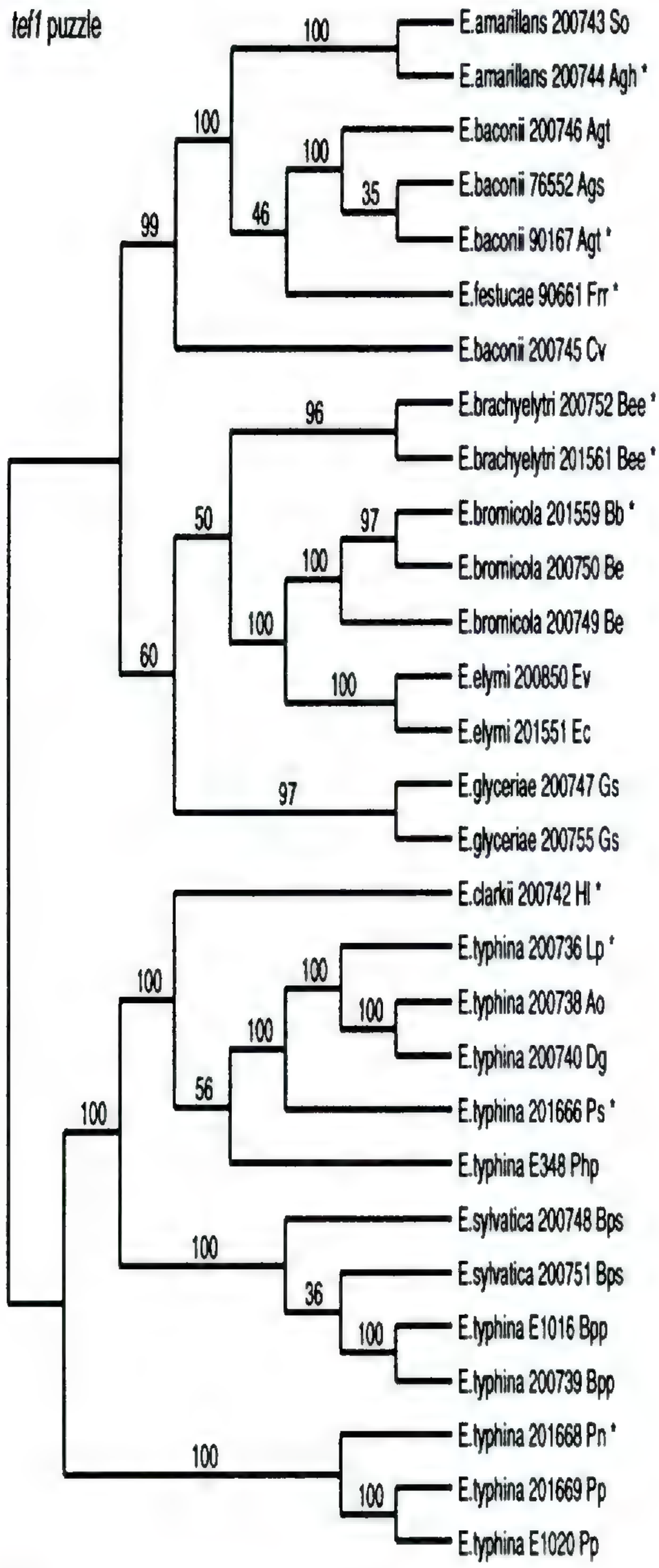


Figure 10. *Epichloë* spp. *tef1* gene tree based on ML puzzle analysis of introns 1–5. Tree is based on a Hasegawa-Kishino-Yano model with ts/tv = 2 (kappa = 3.97409); number of puzzling steps = 1000. Tree shown represents majority-rule consensus, and numbers at branches are estimations of support for each branch.

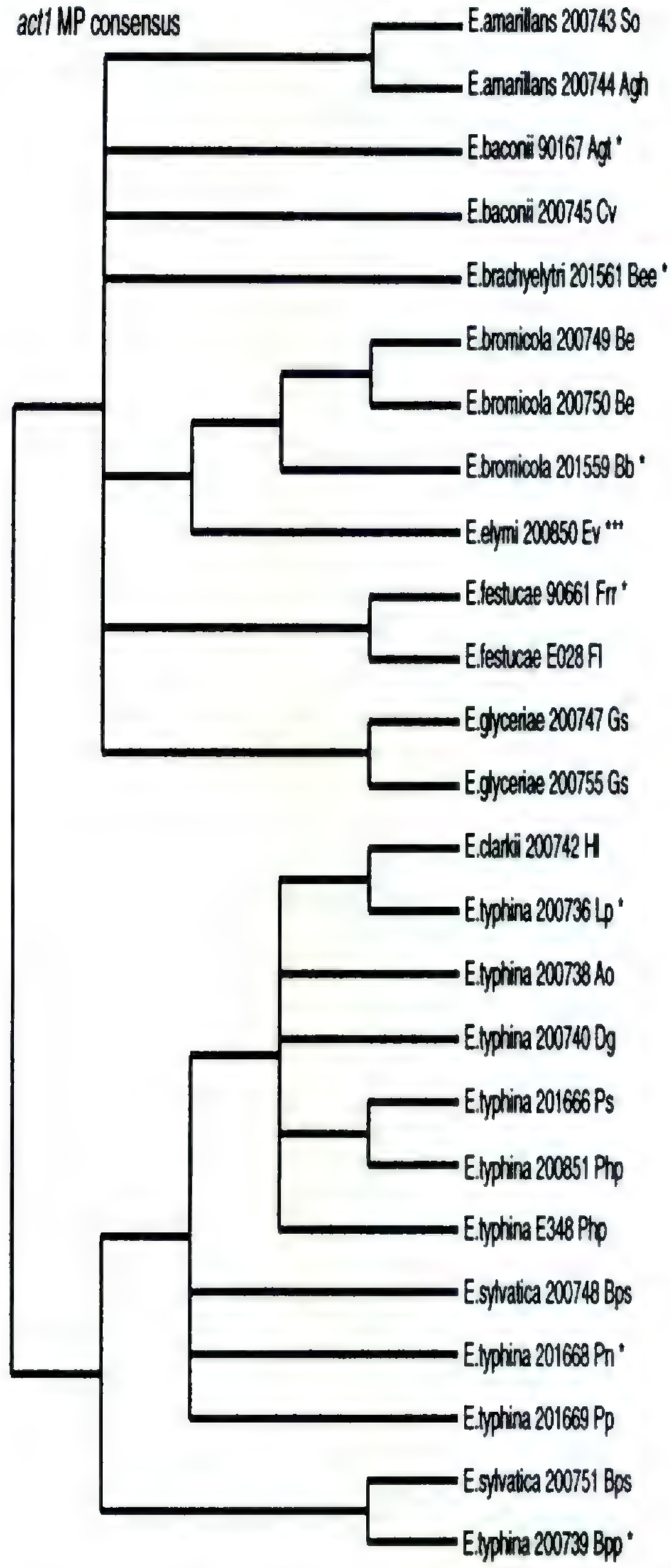


Figure 11. Strict consensus cladogram from the 1946 MP trees derived from a branch-and-bound search of *act1* sequence. Number of parsimony-informative characters = 80; tree length = 149 steps; consistency index = 0.8792; retention index = 0.9430; rescaled consistency index = 0.8291; midpoint root is at the left edge. Asterisks (*) indicate the number of additional isolates (ranging from one to three), sampled from the same host genus, that had identical sequence.

These incongruences and the involvement of these clades were investigated by partition homogeneity tests.

The partition homogeneity test compares phylogenetic information from two genes to determine whether there is statistically significant evidence of incongruence between sequence data sets ($P \leq 0.05$). Since we were interested in reticulation (possible intermating) within the genus *Epichloë*, we removed the outgroup taxa (*C. purpurea* and *Ech. tuberiformis*) from the analysis. Comparison of *act1* with *tef1* resulted in significant incongruence ($P = 0.03$). To determine which taxa may be reticulated, thus the source of incongruence, we removed certain members of the *E. typhina* complex and reanalyzed the data. When all of the crown clade was removed except *E. typhina* from *Dactylis glomerata* and *E. clarkii* from *Holcus lanatus*, and the test was rerun, there was no longer significant incongruence between *act1* and *tef1* trees ($P = 0.20$). Similarly, removal of all *E. typhina* isolates except one from

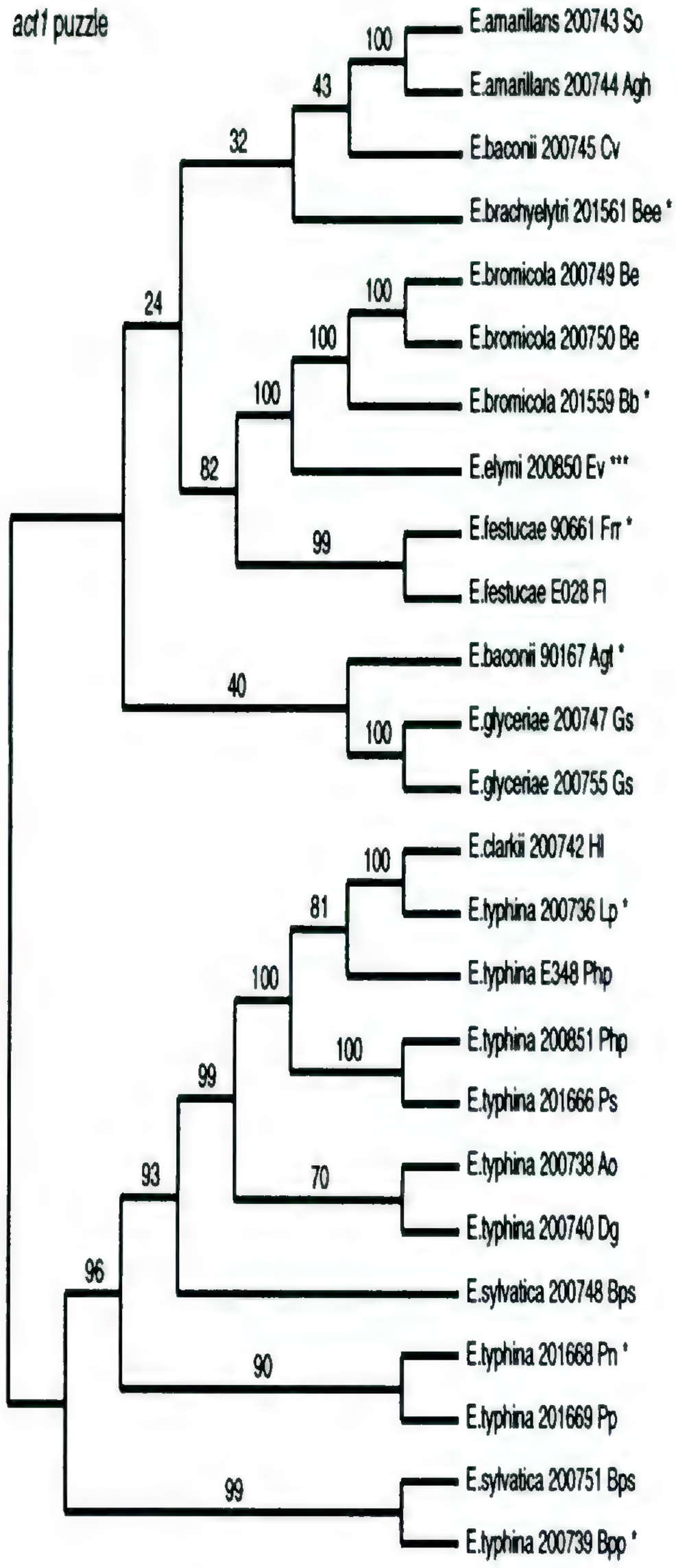


Figure 12. *Epichloë* spp. *act1* gene tree based on ML puzzle analysis of introns 1–4. Tree is based on a Hasegawa-Kishino-Yano model with ts/tv = 2 (kappa = 3.98002); number of puzzling steps = 1000. Tree shown represents majority-rule consensus and numbers at branches are estimations of support for each branch.

D. glomerata, while leaving *E. clarkii* and *E. sylvatica*, gave a nonsignificant test result ($P = 0.47$).

Partition homogeneity analysis of *tub2* and *tef1* gave similar results. When the test included all *Epichloë* taxa, there was a significant incongruence between these two gene sets ($P = 0.01$). Removal of all crown clade isolates except *E. typhina* from *Dactylis glomerata* and *E. clarkii* from *Holcus lanatus* resulted in nonsignificance ($P = 0.71$). Elimination of all *E. typhina* isolates except the *D. glomerata* isolate also resulted in nonsignificance ($P = 0.59$).

Inclusion of all *Epichloë* isolates in the *act1/tub2* test indicated incongruence ($P = 0.01$). In contrast to the other gene comparisons, when the test was run without the crown clade *E. typhina* isolates it still indicated incongruence ($P = 0.02$). However, if taxa from *Poa pratensis*, *P. nemoralis*, and *Brachypodium pinnatum* were also pruned from the test, there was no significant incongruence ($P = 0.23$). Thus, evidence of reticulation in this last comparison was only lost once *E. typhina* isolates from both the crown and basal clades were removed, suggesting reticulation throughout the *E. typhina* complex.

Discussion

Phylogenetic and Biological Species

The main focus of this study was to examine the relationship between biological and phylogenetic species, as well as the role of ecological specialization in genetic isolation of populations of *Epichloë* species. To address these issues, we sought additional genes to compare to the *tub2* gene trees previously inferred (Schardl et al., 1997). Since genetic linkage tests indicated that *tef1*, *act1*, and *tub2* were not closely linked, at least in *E. typhina*, these were considered appropriate for gene tree comparisons. Taxonomic sampling was comparable in scope to our recent *tub2* phylogenetic analysis (Schardl & Leuchtmann, 1999), with all 10 described species of *Epichloë* sensu stricto represented by multiple isolates. The trees inferred from *tef1* and *act1* intron sequences largely corroborated the relationships within the genus *Epichloë* previously elucidated by *tub2* phylogeny (Tsai et al., 1994; Schardl & Leuchtmann, 1999). Analysis of combined data with outgroups also corroborated, with moderate support, the midpoint root inferred for each of the three gene trees. The tree thus rooted divides the genus into two major clades. One clade comprises most of the currently accepted *Epichloë* species, three of which (*E. baconii*, *E. bromicola*, and *E. festucae*) are associated with Eurasian grass hosts (e.g., *Festuca* spp. and *Bromus* spp.), and four (*E. amarillans*, *E. brachyelytri*, *E. elymi*, and *E. glyceriae*) with North American grasses (e.g., *Elymus* spp. and *Agrostis* spp.). This major *Epichloë* clade we refer to as the main group. The other major clade, here termed the *E. typhina* complex, was associated with a broad range of Eurasian grasses and included the described species *E. typhina*, *E. clarkii*, and *E. sylvatica*. For the most part, the main group of *Epichloë* comprised clear-cut phylogenetic species corresponding to established interfertility

groups and morphologically based species. A possible exception, *E. baconii*, is discussed later, as is the possibility of cryptic biological species within morphospecies *E. festucae* and *E. amarillans*. However, the *E. typhina* complex poses the most difficulty in reconciling phylogenetic and biological species concepts.

Biological species can be defined as potentially interbreeding populations that are reproductively isolated from other mating populations (Claridge et al., 1997; Mayr, 1940). Recent descriptions of *Epichloë* species have considered the results of mating tests to identify potentially interbreeding groups (Leuchtmann et al., 1994; Leuchtmann & Schardl, 1998; Schardl & Leuchtmann, 1999; White, 1993, 1994). However, such tests do not necessarily indicate actual interbreeding populations. This is a particular concern if parents are so specialized to different ecological niches, such as hosts, as to preclude survival of their progeny. A phylogenetic approach to identify effectively interbreeding populations has recently been applied to fungi for which the sexual cycle is unknown (Geiser et al., 1998; Burt et al., 1996). The approach is to identify the smallest well-defined phylogenetic units in combined analysis of multiple genes. Such clades are suggested to indicate reproductively isolated populations (Geiser et al., 1998). One assumption is that phylogenetic species, so defined, are equivalent to biological species. In our study we have looked only at known sexual *Epichloë* species and, furthermore, have identified the interfertility groups in the genus. This allows a check on the relationships between phylogenetic and biological species.

We looked for reticulation that would give rise to disparities between tree topologies inferred from different, unlinked genes. Reticulation indicates that either the population has a history of recombination characteristic of an interbreeding gene pool or that much of the extant polymorphism was inherent in the clade's ancestral gene pool. We point out that this analysis cannot indicate how recombination occurs, how often it occurs, or when it last occurred. Genetic tests indicated that the *tef1*, *act1*, and *tub2* genes independently segregated in *Epichloë typhina*, so we considered these genes appropriate for the analysis. Tests of linkage were most feasible in *E. typhina* because this species had sufficient sequence diversity of all three genes for the appropriate primers to be developed and PCR tests to be conducted. An assumption was that these genes are likewise not closely linked in other *Epichloë* clades. We consider this a reasonable assumption because, since they are not linked in *E. typhina*, it is unlikely that all three would be so closely linked in other clades as to affect inferences about genetic reticulation. More importantly, the most apparent conflicts between phylogenetic and biological species were associated with the *E. typhina* complex.

Likely reticulation was identified by conducting partition homogeneity tests both with all taxa included and with most members of potentially reticulating clades removed. Since all three gene trees were obviously concordant for the main *Epichloë* group, we focused on the *E. typhina* complex, where the different gene trees gave different topologies. Not surprisingly, when all taxa were included the test indicated significant conflict between the trees. In two comparisons (*act1/tef1* and *tub2/tef1*) significance was lost when the *E. typhina* crown clade (including *E. clarkii*) was reduced to two representatives. This result strongly suggests that the crown clade comprises or is a component of a reticulated gene pool. Only when the *E. typhina* complex was further pruned by removal of most basal taxa did the test indicate congruent *act1* and *tub2* trees. This suggests that there was also reticulation in the basal taxa, and inspection of the gene trees indicates that the positions of *E. sylvatica* isolates differ in the different trees. The positions of *E. sylvatica* isolates in the three gene trees were a particular surprise, as was the lack of fertility between *E. sylvatica* and *Brachypodium pinnatum*-associated *E. typhina* despite their close relationships (Leuchtmann & Schardl, 1998). It would appear that *E. sylvatica* has emerged as a distinct species from within *E. typhina*, or that *E. sylvatica* has recombined with *E. typhina* in the course of its evolution prior to, or despite, emergence of intersterility barriers.

It is possible that this conflict between biological and phylogenetic species may be a problem of lineage sorting in the *Epichloë typhina* complex. Lineage sorting arises due to polymorphisms present in an ancestral mating population from which different alleles are fixed in extant lineages, and can result in incorrect phylogenetic inferences being drawn as to the true relationships between extant lineages (Lyons-Weiler & Milinkovitch, 1997). Another possibility is that there has been parasexual recombination involving *E. typhina* and *E. sylvatica* (Caten, 1981). This is plausible considering the origins of many asexual endophytes as hybrids of multiple *Epichloë* species. However, the hybrid asexual species generally have large portions of the genomes of their ancestors, thus containing multiple copies of most genes (Kuldau et al., 1999). There is no indication of this in *Epichloë* species from analysis of *tub2*, *tef1*, and *act1*, though occa-

sional multiband phenotypes have been observed in isozyme studies of *E. typhina* (Leuchtmann & Schardl, 1998). Even if parasexual recombination occurs in the *E. typhina* complex, the close relationship between *E. sylvatica* ATCC 200751 and *E. typhina* from *Brachypodium pinnatum* is unexpected in that all three gene trees give this same relationship, and isozyme analysis gives a similar result (Leuchtmann & Schardl, 1998). In stark contrast, *E. typhina* from *Bp. pinnatum* did not share any identical or highly similar gene sequences with *E. typhina* isolates from any other host, as would have been expected from either sexual or parasexual recombination. Although we cannot exclude either lineage sorting or parasexual recombination to explain the apparent paraphyly of *E. typhina* to *E. sylvatica,* as well as polyphyly of both biological species, these relationships nevertheless amount to a conflict between the phylogenetic and biological species concepts.

With the exception of the *E. typhina* complex, most phylogenetic species of *Epichloë* had little genetic diversity, and there is little or no evidence of gene flow (reticulation) between phylogenetic species. Previous mating test results (Schardl & Leuchtmann, 1999) have shown that many phylogenetic species in the main *Epichloë* group also conform to a biological species concept; there was intermating only between isolates from the same or closely related hosts, often within one host genus. Yet, even in the main group there were some cases where phylogenetic species did not precisely predict interfertility groups or vice versa. *Epichloë baconii* isolates from host genera *Agrostis* and *Calamagrostis,* though capable of intermating, never definitively grouped in a monophyletic clade in any gene phylogram (this was also the observation based on allozyme cluster analysis; Leuchtmann & Schardl, 1998). However, it is possible that whenever *Agrostis*- and *Calamagrostis*-associated populations intermate in nature their progeny are lost due to lack of a compatible host. Such a situation would isolate the populations into different biological species despite their observed interfertility in experimental matings. More sampling of the *E. baconii* populations is needed to assess if they actually represent distinct biological species or, alternatively, if this is another conflict between phylogenetic and biological species concepts.

The converse situation apparently arises with *Epichloë amarillans* and *E. festucae.* An *E. amarillans* isolate on *Sphenopholis obtusata* had limited or no interfertility with an isolate from *Agrostis hiemalis.* Although mating proceeded as expected, progeny showed no evidence of genetic contribution from the male. Though this does not preclude limited genetic exchange, the result suggests that these host-associated populations have a degree of genetic isolation. Since phylogenetic analysis placed these populations into distinct clades they may indeed represent different biological species. Likewise, *E. festucae* from *Festuca rubra* failed to mate with an isolate from *Koeleria cristata* even though they had identical gene sequences. This test must be considered preliminary pending reciprocal mating tests. Nevertheless, the result points up a potential limitation that if sequence evolution does not adequately keep pace with biological speciation it may be difficult to identify species on a strictly phylogenetic basis.

GENE FLOW WITHIN A BIOLOGICAL SPECIES

The relationships among host-associated populations in the *Epichloë typhina* complex is intriguing. In keeping with previous results from isozyme analysis (Leuchtmann & Schardl, 1998) we found that certain host grasses harbor genetically diverse endophyte populations, whereas other hosts harbor populations of genetically similar individuals. Among the more diverse are the *E. typhina* populations in *Dactylis glomerata* and *Phleum pratense,* as well as *E. sylvatica* from *Brachypodium sylvaticum.* In contrast, isolates from other host-associated fungal populations had similar or identical allozyme profiles and DNA sequences. Little or no sequence variation was observed within *E. typhina* populations in many of its grass hosts: *Anthoxanthum odoratum* (based on *tub2*), *Brachypodium pinnatum* (*tub2* and *tef1*), *Lolium perenne* (*tub2, tef1,* and *act1*), *Poa nemoralis* (*tub2, tef1,* and *act1*), and *P. pratensis* (*tub2*); as well as among *E. clarkii* from *Holcus lanatus* (*tub2* and *tef1*). Since most of these host-associated populations are currently represented by a small sample size it is unclear whether they might be substantially more diverse than so far indicated. Still, the genetic uniformity of *E. clarkii* seems well substantiated because this species is represented by five isolates from Britain and Switzerland, and both mating types are included. Even though most *E. clarkii* isolates are readily interfertile with *E. typhina* on several other hosts, *E. clarkii* seems to maintain genetic and even morphological distinction from the rest of the *E. typhina* complex (White, 1993; Leuchtmann & Schardl, 1998). However, the position of *E. clarkii* relative to the various *E. typhina* isolates is not consistent among gene trees, so that *E. clarkii* does not fit the criterion for a phylogenetic species. It is possible that *E. typhina* on other grasses—such as *Dactylis*

glomerata—actually mates with *E. clarkii* in nature, but that the progeny do not colonize or survive on *Holcus lanatus*, host grass for *E. clarkii*. Perhaps only *E. clarkii* × *E. clarkii* progeny can colonize this host, leading to extreme inbreeding but not necessarily genetic isolation. Thus, *E. clarkii* may contribute to the gene pool of certain *E. typhina* populations, but *E. typhina* rarely or never contributes to the *E. clarkii* (i.e., *H. lanatus*-associated) gene pool. The other low-diversity host-associated populations may have similar relationships to the more diverse ones.

Is incipient speciation occurring in the *Epichloë typhina* complex? One might expect speciation based on host-associated populations given the strong association of other *Epichloë* species with individual host genera or tribes. *Epichloe sylvatica* appears to be an example, since it is restricted to one host, *Brachypodium sylvaticum*. Yet even if such species emerge, it appears that they will not correspond to such clear-cut phylogenetic clades as do the other *Epichloë* species. This, also, is illustrated by the apparent polyphyly of *E. sylvatica*. Overall, no phylogenetic species obviously emerge from host-associated populations in the *E. typhina* complex. However, it appears that the crown clade of *E. typhina/E. clarkii* may have effectively speciated from the more basal clades. All indications of reticulation were either within the crown clade or among the basal clades, but not between the crown and basal clades. This suggests that the crown clade is an incipient species that has not fully established genetic barriers to mating outside the clade (many matings between basal and crown clade members are fertile; Leuchtmann & Schardl, 1998). Furthermore, even though one genus (*Poa*) is represented among hosts of both basal and crown clades, the different clades consistently comprise isolates from different host species. *Epichloë* species in the crown clade infect *P. silvicola* and *P. trivialis*, whereas members of the more basal clades infect *P. pratensis* and *P. nemoralis*.

PREVAILING TRANSMISSION MODES AND EVOLUTION

The apparently different evolutionary processes between the *Epichloë typhina* complex and the "main group" containing the other seven *Epichloë* species may relate to their different host interactions, namely whether they are more benign and vertically transmissible or more virulent (antagonistic) and reliant on horizontal transmission. Most members of the *E. typhina* complex are antagonistic (strong chokers) in that they shut down almost all host seed production. They must therefore rely on horizontal transmission. As discussed above, speciation in this complex is not clear cut. In contrast, most of the species in the main group balance vertical and horizontal transmission, and, as partial chokers, have more benign or even mutualistic effects on host plants. In this group speciation follows a cladistic stereotype and most species boundaries are unambiguous.

Species in the main *Epichloë* group with substantial seed transmission include *E. brachyelytri*, *E. amarillans*, *E. festucae*, and *E. elymi* (Leuchtmann & Schardl, 1998; Leuchtmann et al., 1994; Schardl & Leuchtmann, 1999). The situation for *E. bromicola* is unique in that it is a strong choker on *Bromus erectus*, but is strictly seed transmitted on other *Bromus* species (Leuchtmann & Schardl, 1998). It is possible that *E. bromicola* has both transmission modes on an unidentified host. Only two main group species, *E. glyceriae* and *E. baconii*, are strong chokers for which vertical transmission is unknown. The deep rooting of *E. brachyelytri* (based on combined sequence analysis; Fig. 3) and the preponderance of vertically transmitted species suggest that this is an ancestral trait of the main *Epichloë* group. We previously proposed that the loss of seed transmissibility in *E. glyceriae* might have been associated with a host jump, since without this fungal species the relationships among the main *Epichloë* group largely reflect host relationships (Schardl et al., 1997). However, since the relevant branches were unresolved in *tef1* and *act1* trees, it remains possible that *E. glyceriae* and the rest of the main group have tracked the evolutionary paths of their hosts.

Even with inconsistent phylogenetic tracking it is likely that host specialization played a role in *Epichloë* speciation. Since vertical transmission provides a dependable route for dissemination there is little benefit expected for broadening host range. This is particularly true if there is a tradeoff between broad host range and the balance of vertical and horizontal transmission in these fungi. We have suggested that such a balance of host and fungal development is unlikely to survive host jumps, and that this is why the more mutualistic *Epichloë* species seem to track host phylogeny (Schardl et al., 1997). Here we suggest that this is also why there are clear boundaries between host-associated species in the main *Epichloë* group. As further evidence that benign or mutualistic interactions have promoted host-based speciation, the phylogenetic pattern is substantially different for the more antagonistic species, especially the *E. typhina* complex. Models of host-parasite interactions predict that high virulence levels (antagonism) should cor-

relate with rapid contagious spread (Yamamura, 1993). No comparative study has been done on transmission rates of *Epichloë* species. However, horizontal transmission of *E. typhina* appears to be very rapid in *Dactylis glomerata* populations, as indicated by a recent epidemic in Oregon (Pfender & Alderman, 1999). We speculate that high virulence and reliance on horizontal transmission selects for broad host range and limits speciation in the *E. typhina* complex. There are two reasons why *E. typhina* may be particularly subject to this kind of selection. First, since this species depends heavily on horizontal transmission the availability of hosts within a short range of a parent stroma (specifically, the dispersal area for ascospores) is important for its propagation. Second, it can participate in reducing the host population, and indeed it will be a factor in the reduction of available hosts by reducing flowering. Whenever local host populations decline either by effects of *E. typhina* or other factors, there would be increased selection on the fungus to colonize other hosts. Such a selective effect is not expected for more benign *Epichloë* species because their assured vertical dissemination obviates any immediate reliance on horizontal transmission to neighboring plants.

ORIGINS OF THE MUTUALISTIC ENDOPHYTES

The main group of *Epichloë* species represents the emergence of a mechanism to maximize mutual benefit to host and symbiont. This mechanism is a balanced interaction whereby individual plant-fungus symbiota can simultaneously exhibit sexual propagation of both partners. Although the necessary components of such interactions are represented among related Clavicipitaceae (Clay & Jones, 1984; Kover & Clay, 1998), it is only in the main *Epichloë* group that such a balance is consistently evident (Schardl & Clay, 1997). In contrast, the more antagonistic effects of the *E. typhina* complex are similar to those of many other plant-associated Clavicipitaceae, particularly *Balansia* species (Diehl, 1950; Kuldau et al., 1997). Furthermore, most of the *E. typhina* complex, like most *Balansia* species, fail to transmit in host seeds. Although *E. typhina* on *Poa nemoralis* can transmit vertically, it tends to choke the vast majority of tillers and thereby severely limit both host seed production and its potential for vertical transmission. Likewise, although *E. sylvatica* was previously considered to have balanced host interactions, more detailed examination has indicated that stroma-forming genotypes are actually strong chokers. What were previously thought to be balanced interactions were simultaneous infections, whereby individual *Brachypodium sylvaticum* plants had two endophytes, one sexual (*E. sylvatica*) and the other asexual (Meijer & Leuchtmann, 1999). Though closely related to *E. sylvatica*, these asexual endophytes represent the opposite extreme in that they have sacrificed all capacity for horizontal spread and are strictly vertically transmitted. Although this may well benefit the host, it is nevertheless apparent that the balanced interactions characterizing much of the *Epichloë* main group species are absent in the *E. typhina* complex as they are among other Clavicipitaceae.

Symbioses of asexual endophytes with grasses represent a third class of fungal/host interactions distinct from the balanced or antagonistic associations involving sexual *Epichloë* species. Many grasses in subfamily Pooideae possess asexual derivatives of *Epichloë* species, which, by current taxonomic convention, are classified in a distinct genus in the Fungi Imperfecti. Several such species have been formally described and placed in genus *Neotyphodium* (Glenn et al., 1996). These endophytes are often beneficial to their hosts and cause no disease, so they are commonly regarded as mutualists. We are currently analyzing *tub2*, *tef1*, and *act1* noncoding regions for several *Neotyphodium* species, some formally described and others remaining to be described. Results from this *tub2* analysis indicate that several have hybrid origin (Schardl et al., 1994; Tsai et al., 1994; Moon et al., 2000), a conclusion also supported by analysis of *tef1* and *act1* genes (K.D.C. & C.L.S., unpublished obs.). As we expand the number of genes and species analyzed it is becoming apparent that hybrid origins are very common among *Neotyphodium* spp., though not universal. For example, the asexual *N. coenophialum*, a common endophyte of *Lolium arundinaceum* (= *Festuca arundinacea*), has three *Epichloë* species in its pedigree (Tsai et al., 1994); whereas *N. lolii*, the most common endophyte of *Lolium perenne*, seems to be a direct descendant of *E. festucae*. Those *Epichloë* species involved in known hybridizations span the phylogenetic diversity of the genus, including *E. typhina*, *E. bromicola*, *E. elymi*, *E. amarillans*, *E. festucae*, and a relative of *E. baconii*. The hybridizations have led to diploidy, polyploidy, or extensive aneuploidy in most *Neotyphodium* species studied to date, and for this and other reasons are likely somatic events rather than hybrid matings (Schardl et al., 1994; Kuldau et al., 1999). Thus, traditional species concepts are violated, albeit in different ways, both by endophytes that only trans-

mit vertically and those that only transmit horizontally.

Conclusions

The most striking result from our phylogenetic studies of *Epichloë* species and their asexual relatives is that different evolutionary patterns are associated with different modes of transmission to new host plants. A cladistic pattern, usually assumed to describe speciation in most organisms, was only apparent for one of the two major lineages in the *Epichloë* phylogeny. Surprisingly, this was the group that had an exquisite balance of horizontal and vertical transmission uncharacteristic of the fungal family and, indeed, uncharacteristic of most pathogens and symbionts. In contrast, cladistic evolution is less evident both for strictly vertically transmitted and strictly horizontally transmitted species. Interspecific hybridization is often evident in the origins of the vertically transmitted, asexual symbionts (Schardl et al., 1994; Tsai et al., 1994). Further, the *E. typhina* complex—dominated by strains that only transmit horizontally—lacks clear-cut genetically isolated groups (this study). We speculate that high virulence and dependence on horizontal transmission might counteract host specialization and, in turn, promote intraspecies diversity and delay speciation. Delayed speciation should amplify lineage sorting problems that cause conflict between the biological and phylogenetic species concepts, as discussed above. If a reliance on horizontal transmission delays speciation as we speculate, an obvious prediction is that other highly virulent pathogens will exhibit conflicts between biological and phylogenetic species. To our knowledge, this has not yet been tested. A good relationship between these species concepts was noted for the *Gibberella fujikuroi* group (O'Donnell et al., 1998), but these fungi also exhibit both horizontal and vertical transmission (Bacon & Hinton, 1996). Detailed phylogenies and population surveys are clearly needed for more virulent pathogens, organisms that cause the most problems in human health and the food supply.

Literature Cited

Al Samarrai, T. H. & J. Schmid. 2000. A simple method for extraction of fungal genomic DNA. Lett. Appl. Microbiol. 30: 53–56.

Arechavaleta, M., C. W. Bacon, C. S. Hoveland & D. E. Radcliffe. 1989. Effect on the tall fescue endophyte on plant response to environmental stress. Agron. J. 81: 83–90.

Bacon, C. W. & D. M. Hinton. 1996. Symptomless endophytic colonization of maize by *Fusarium moniliforme*. Canad. J. Bot. 74: 1195–1202.

———, J. K. Porter, J. D. Robbins & E. S. Luttrell. 1977. *Epichloë typhina* from toxic tall fescue grasses. Appl. Environm. Microbiol. 34: 576–581.

Burt, A., D. A. Carter, G. L. Koenig, T. J. White & J. W. Taylor. 1996. Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. Proc. Natl. Acad. Sci. U.S.A. 93: 770–773.

Byrd, A. D., C. L. Schardl, P. J. Songlin, K. L. Mogen & M. R. Siegel. 1990. The β-tubulin gene of *Epichloë typhina* from perennial ryegrass (*Lolium perenne*). Curr. Genet. 18: 347–354.

Caten, C. E. 1981. Parasexual processes in fungi. Pp. 191–214 *in* K. Gull & S. G. Oliver (editors), The Fungal Nucleus. Cambridge Univ. Press, Cambridge.

Claridge, M. F., H. A. Dawah & M. R. Wilson. 1997. Practical approaches to species concepts for living organisms. Pp. 1–15 *in* M. F. Claridge, H. A. Dawah & M. R. Wilson (editors), Species: The Units of Biodiversity, 1st ed. Chapman & Hall, London.

Clay, K. & J. P. Jones. 1984. Transmission of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) by cleistogamous seed of *Danthonia spicata* (Gramineae). Canad. J. Bot. 62: 2893–2898.

Diehl, W. W. 1950. *Balansia* and the Balansiae in America. Agric. Monogr. U.S.D.A. 4.

Felsenstein, J. 1988. Phylogenies from molecular sequences: Inference and reliability. Annual Rev. Genet. 22: 521–565.

Fidel, S., J. H. Doonan & N. R. Morris. 1988. *Aspergillus nidulans* contains a single actin gene which has unique intron locations and encodes a γ-actin. Gene 70: 283–293.

[GCG] Genetics Computer Group. 1996. Wisconsin Package. Version 9.0. Published by the authors, Madison, Wisconsin.

Geiser, D. M., J. I. Pitt & J. W. Taylor. 1998. Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*. Proc. Natl. Acad. Sci. U.S.A. 95: 388–393.

Glenn, A. E., C. W. Bacon, R. Price & R. T. Hanlin. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia 88: 369–383.

Hillis, D. M. 1987. Molecular versus morphological approaches to systematics. Annual Rev. Ecol. Syst. 18: 23–42.

Kennedy, C. W. & L. P. Bush. 1983. Effect on environmental and management factors on the accumulation of N-acetyl and N-formyl loline alkaloids in tall fescue. Crop. Sci. 23: 547–552.

Kover, P. X. & K. Clay. 1998. Trade-off between virulence and vertical transmission and the maintenance of a virulent plant pathogen. Amer. Naturalist 152: 165–175.

Kuldau, G. A., J-S. Liu, J. F. White, Jr., M. R. Siegel & C. L. Schardl. 1997. Molecular systematics of Clavicipitaceae supporting monophyly of genus *Epichloë* and form genus *Ephelis*. Mycologia 89: 431–441.

———, H.-F. Tsai & C. L. Schardl. 1999. Genome sizes of *Epichloë* species and anamorphic hybrids. Mycologia 91: 776–782.

Leuchtmann, A. & C. L. Schardl. 1998. Mating compatibility and phylogenetic relationships among two new species of *Epichloë* and other congeneric European species. Mycol. Res. 102: 1169–1182.

———, ——— & M. R. Siegel. 1994. Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. Mycologia 86: 802–812.

Lyons-Weiler, J. & M. C. Milinkovitch. 1997. A phylogenetic approach to the problem of differential lineage sorting. Molec. Biol. Evol. 14(9): 968–975.

Margulis, L. 1991. Evolution of eukaryotes. Amer. Scientist 79: 187–188.

Mayr, E. 1940. Speciation phenomena in birds. Amer. Naturalist 74: 249–278.

Meijer, G. & A. Leuchtmann. 1999. Multistrain infections of the grass *Brachypodium sylvaticum* by its fungal endophytes *Epichloë sylvatica*. New Phytol. 141: 355–368.

Moon, C. D., B. Scott, C. L. Schardl & M. J. Christensen. 2000. Evolutionary origins of *Epichloë* endophytes from annual ryegrasses. Mycologia 92: 1103–1118.

Moritz, C. & D. M. Hillis. 1996. Molecular systematics: Context and controversies. Pp. 1–13 *in* D. M. Hillis, C. Moritz & B. K. Mable (editors), Molecular Systematics (2nd ed.). Sinauer Associates, Sunderland, Massachusetts.

O'Donnell, K., E. Cigelnik & H. I. Nirenberg. 1998. Molecular systematics and phytogeography of the *Gibberella fujikuroi* species complex. Mycologia 90: 465–493.

———, H. C. Kister, E. Cigelnik & R. C. Ploetz. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene geneologies. Proc. Natl. Acad. Sci. U.S.A. 95: 2044–2049.

Pfender, W. F. & S. C Alderman. 1999. Geographical distribution and incidence of orchardgrass choke, caused by *Epichloë typhina*, in Oregon. Plant Dis. 83: 754–758.

Sampson, K. 1933. The systemic infection of grasses by *Epichloë typhina* (Pers.) Tul. Trans. Brit. Mycol. Soc. 18: 30–47.

Schardl, C. L. & Z. An. 1993. Molecular biology and genetics of protective fungal endophytes of grasses. Pp. 191–212 *in* J. K. Setlow (editor), Genetic Engineering, Principles and Methods, Vol. 15. Plenum Press, New York.

——— & K. Clay. 1997. Evolution of mutualistic endophytes from plant pathogens. Pp. 221–238 *in* G. C. Carroll & P. Tudzynski (editors), The Mycota V: Plant Relationships, Part B. Springer-Verlag, Berlin.

——— & A. Leuchtmann. 1999. Three new species of *Epichloë* symbiotic with North American grasses. Mycologia 91: 95–107.

——— & T. D. Phillips. 1997. Protective grass endophytes: Where are they from and where are they going? Plant Dis. 81: 430–438.

——— & H. H. Wilkinson. 2000. Hybridization and cospeciation hypotheses for the evolution of grass endophytes. Pp. 63–83 *in* C. W. Bacon & J. F. White, Jr. (editors), Microbial Endophytes. Marcel Dekker, New York & Basel.

———, A. Leuchtmann, K.-R. Chung, D. Penny & M. R. Siegel. 1997. Coevolution by common descent of fungal symbionts (*Epichloë* spp.) and grass hosts. Molec. Biol. Evol. 14: 133–143.

———, ———, H. F. Tsai, M. A. Collett, D. M. Watt & D. B. Scott. 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloë typhina*. Genetics 136: 1307–1317.

Siegel, M. R., M. C. Johnson, D. R. Varney, W. C. Nesmith, R. C. Buckner, L. P. Bush, P. B. Burrus, II, T. A. Jones & J. A. Boling. 1984. A fungal endophyte in tall fescue: Incidence and dissemination. Phytopathology 74: 932–937.

———, U. Jalfors, G. C. M. Latch & M. C. Johnson. 1987. Ultrastructure of *Acremonium coenophialum*, *Acremonium lolii*, and *Epichloë typhina* endophytes in host and non-host *Festuca* and *Lolium* species of grasses. Canad. J. Bot. 65: 2357–2367.

Strimmer, K. & A. von Haeseler. 1996. Quartet puzzling: A quartet maximum-likelihood method for reconstructing tree topologies. Molec. Biol. Evol. 13(7): 964–969.

Swofford, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

———, G. J. Olsen, P. J. Waddell & D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *in* D. M. Hillis, C. Moritz & B. K. Mable (editors), Molecular Systematics (2nd ed.). Sinauer Associates, Sunderland, Massachusetts.

Thomas, W. K. & T. D. Kocher. 1993. Sequencing of polymerase chain reaction-amplified DNAs. Meth. Enzymol. 224: 391–399.

Tsai, H.-F., J. S. Liu, C. Staben, M. J. Christensen, G. C. M. Latch, M. R. Siegel & C. L. Schardl. 1994. Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloë* species. Proc. Natl. Acad. Sci. U.S.A. 91: 2542–2546.

Tudzynski, P., K. B. Tenberge & B. Oeser. 1995. *Claviceps purpurea*. Pp. 161–187 *in* K. Kohmoto, U. S. Singh & R. P. Singh (editors), Pathogenesis and Host Specificity in Plant Diseases: Histopathological, Biochemical, Genetic and Molecular Bases, Vol. 2, Eukaryotes. Pergamon, Tarrytown, New York.

White, J. F., Jr. 1993. Endophyte-host associations in grasses. XIX. A systematic study of some sympatric species of *Epichloë* in England. Mycologia 85: 444–455.

———. 1994. Endophyte-host associations in grasses. XX. Structural and reproductive studies of *Epichloë amarillans sp. nov.* and comparisons to *E. typhina*. Mycologia 86: 571–580.

——— & T. L. Bultman. 1987. Endophyte-host associations in forage grasses. VIII. Heterothallism in *Epichloë typhina*. Amer. J. Bot. 74: 1716–1721.

Yamamura, N. 1993. Vertical transmission and evolution of mutualism from parasitism. Theoret. Populat. Biol. 44: 95–109.

THE COEVOLUTION OF BACTERIAL ENDOSYMBIONTS AND PHLOEM-FEEDING INSECTS[1]

Nancy A. Moran[2]

ABSTRACT

Insects that feed on plant sap depend on symbiotic bacteria for nutrients that are not present in the diet. These bacteria live within host cells and are transmitted from mother to offspring. The symbionts of aphids, in the genus *Buchnera*, are the best characterized of insect endosymbionts. They result from an ancient infection of a common ancestor of modern aphids, and they possess genes underlying pathways for production of essential amino acids that are rare in the phloem sap diet of aphids. Thus these bacteria appear to be highly coadapted with hosts and to have evolved as mutualists for millions of years. Nonetheless, *Buchnera* and other endosymbionts possess some genetic traits that appear to be deleterious to both hosts and, ultimately, the bacterial symbionts themselves. The most likely basis for these traits is the fixation of slightly deleterious mutations in the context of genetic drift in these bacterial populations, which possess small genetic population sizes relative to many free-living bacteria. Investigations during the next few years will reveal the extent of convergence among independently derived symbionts of different insect families and the extent to which symbionts are adapted to species-specific aspects of their host's ecology and nutrition.

Key words: aphid, *Buchnera*, coevolution, endosymbiont, mutualism, phloem sap, phylogenetics.

INTRODUCTION: PHLOEM-FEEDING AND ANIMAL NUTRITION

Among the major groups of organisms, animals stand out for their limited biosynthetic capabilities. Many vitamins and amino acids cannot be synthesized by animals, which must ingest each of these compounds in the diet. As a result, dietary specialization imposes the risk of nutritional deficiencies.

Several groups of insects feed only on the phloem sap of plants. Because tapping into a single phloem sieve element allows access to nutrients from throughout the plant without moving, this feeding mode is potentially rewarding. Furthermore, phloem sap of plants is rich in sugars and contains free amino acids. However, the nutrient profile of phloem is not favorable to animal growth. Although nitrogen is present in the form of free amino acids, there is a deficit of essential amino acids, the set required in animal diets (Fig. 1; Sandström & Moran, 1999). Whereas plant and animal proteins show similar amino acid abundance profiles, phloem sap contains primarily a few nonessential amino acids, which are transaminated as needed for protein synthesis at the site of growth. Thus, the reproduction and development of phloem feeders may be limited by certain essential amino acids, causing excess nonessential amino acids to be excreted as waste (Sandström & Moran, 1999). In addition, phloem may be deficient in some vitamins.

Phloem-feeding insects have adopted a common solution to nutritional deficiencies: association with bacterial endosymbionts that have the capacity for biosynthesis of required amino acids (P. Baumann et al., 1999). These bacteria are confined to specialized host cells called bacteriocytes (or mycetocytes) that together form an organ called a bacteriome (Moran & Telang, 1998). The bacteria are transmitted maternally with progeny inoculated before oviposition or birth. Bacteriocyte associates appear to be present in all strict phloem-feeders, including most members of the clade corresponding to suborder Sternorrhyncha and many members of the suborder Auchenorrhyncha. These two suborders within the Hemiptera are often lumped as Homoptera, although not a monophyletic group (von Dohlen & Moran, 1995). The Sternorrhyncha includes aphids (Aphidoidea), scale insects (Coccoidea), whiteflies (Aleyrodoidea), and psyllids (Psylloidea) (Fig. 2). Current understanding of the genetics and evolution of the aphid-symbiont association is more extensive than for any other animal endosymbiosis. More limited knowledge for psyllids, whiteflies, and some scale insects suggests

[1] Elizabeth Bernays gave useful comments on a draft of this manuscript. The writing and much of the work reviewed were supported by NSF grants DEB 9815413 and DEB 9978518 to NM.

[2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721, U.S.A.

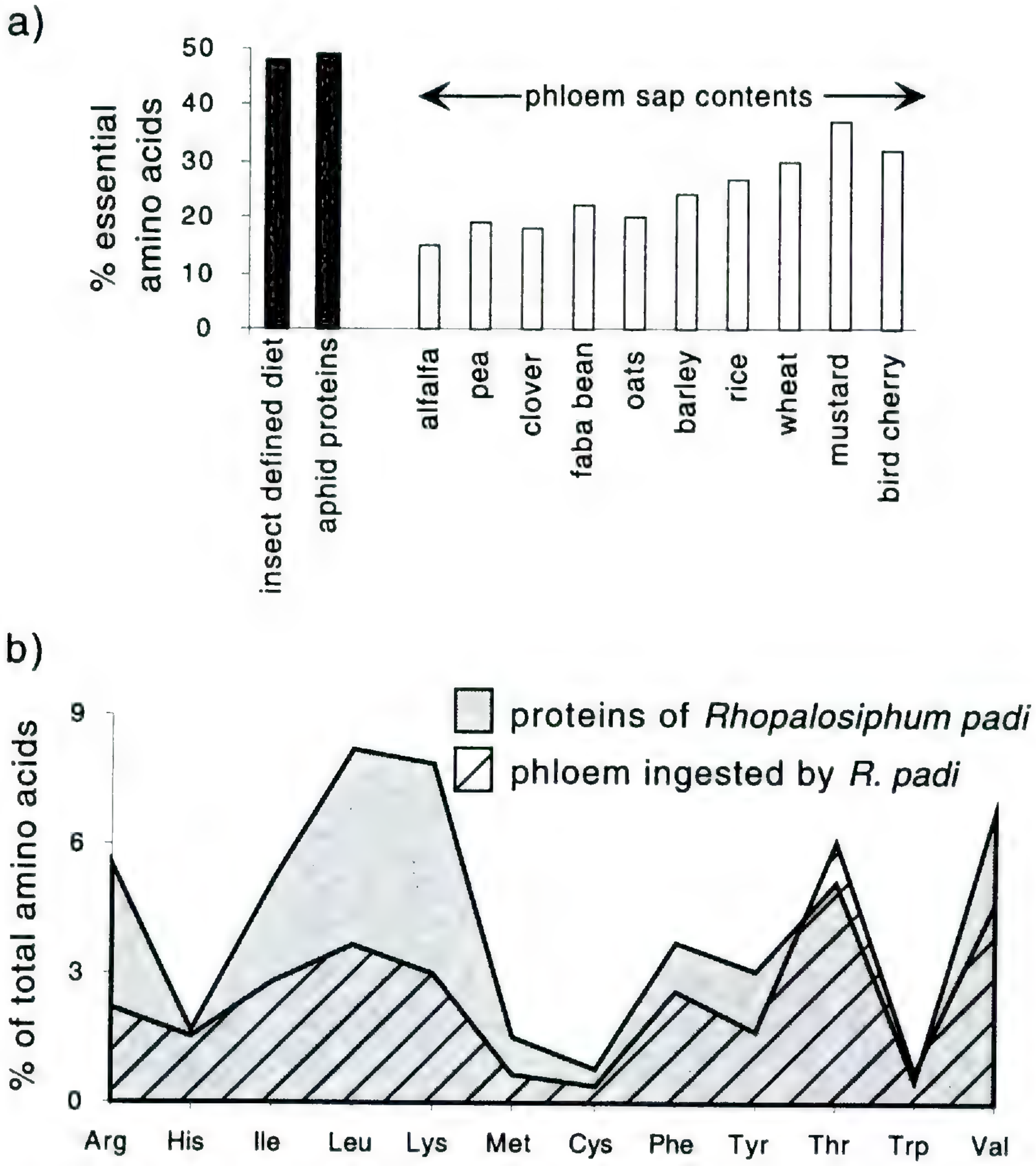


Figure 1. Quality of phloem sap as a diet for aphids. —a. Proportion of essential amino acids in aphid proteins, insect-defined diets, and phloem sap of various plants as sampled from aphid stylets. In phloem sap, a higher proportion of amino acids are nonessential. —b. Proportions of individual essential amino acids of total amino acids in the diet and body proteins of the aphid *Rhopalosphium padi*. Most essential amino acids are deficient relative to requirements for protein synthesis. (Although amino acid profiles of proteins are roughly representative of dietary requirements, some, including tryptophan, are required in higher concentrations due to use in other endproducts or due to differences in efficiency of absorption). Data are summarized from Sandström and Moran (1999) for various studies based on phloem sap ingested by different aphid species on several angiosperm species (alfalfa = *Medicago sativa* L.; pea = *Pisum sativum* L.; clover = *Trifolium pratense* L.; faba bean = *Vicia faba* L.; oats = *Avena sativa* L.; barley = *Hordeum vulgare* L.; rice = *Oryza sativa* L.; wheat = *Triticum aestivum* L.; mustard = *Brassica juncea* (L.) Czern.; bird cherry = *Prunus padus* L.).

that similar patterns of evolution will extend at least to these groups, and some parallels have been observed in other invertebrate endosymbioses, including cockroaches (Bandi et al., 1995), carpenter ants (Schröder et al., 1996), tsetse flies (Chen et al., 1999), and bivalves (Peek et al., 1998).

In this paper, I present an overview of the current knowledge of the coevolution of endosymbionts with hosts in the Sternorrhyncha, especially aphids.

Biological Background

Aphid primary endosymbionts are classified as *Buchnera aphidicola* (Munson et al., 1991a) and belong to the gamma-Proteobacteria, near *Escherichia coli* and other enterics (Munson et al., 1991b). *Buchnera* lives within maternal cells called bacteriocytes, and infection occurs within the mother's body, during the egg or embryo stage (Buchner, 1965). Increase in bacterial numbers during development closely tracks the increase in aphid body size (Baumann & Baumann, 1994). Nutritional studies suggest that *Buchnera* provisions the host with certain amino acids (Douglas, 1998), and genetic studies have confirmed that the enzymatic pathways for amino acid biosynthesis are retained and modified to allow increased pro-

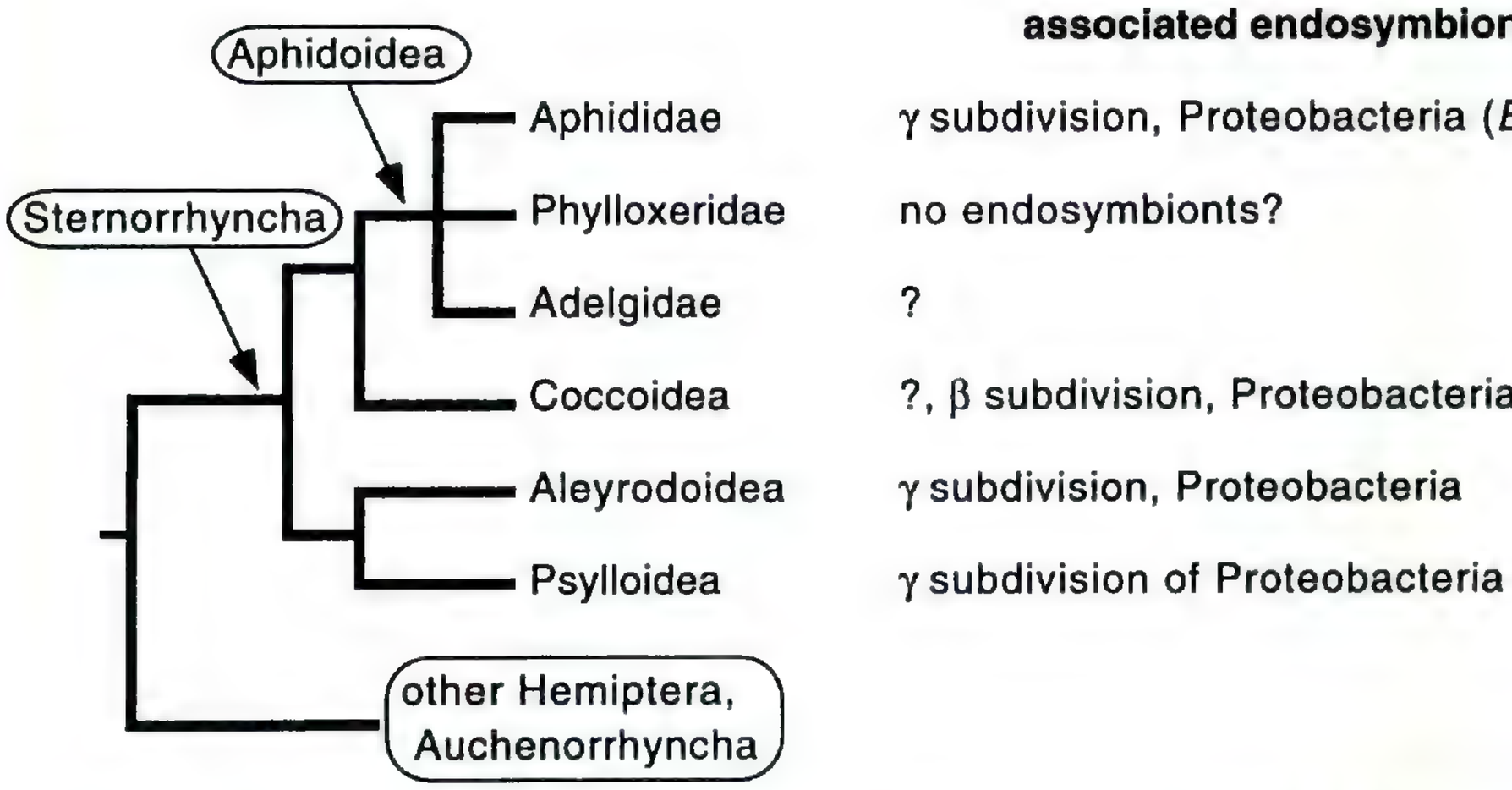


Figure 2. Phylogenetic relationships within the Sternorrhyncha (von Dohlen & Moran, 1995) and affiliations, where known, of bacteriocyte-associated or "primary" symbionts of major groups (Buchner, 1965; Munson et al., 1991b; Clark et al., 1993; Spaulding & von Dohlen, 1998; Thao et al., 2000a).

duction of end products (Lai et al., 1994; Bracho et al., 1995).

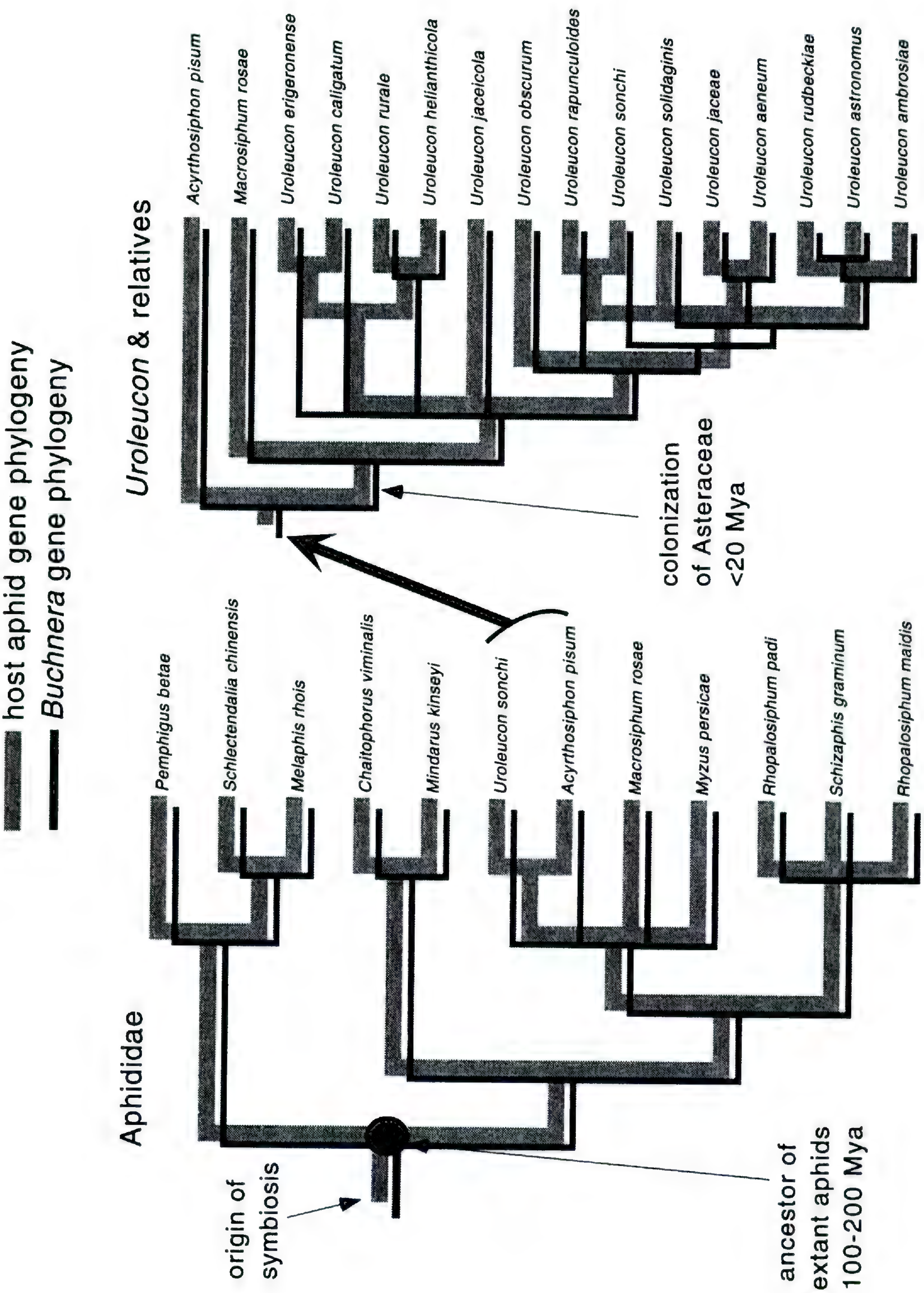
Other Sternorrhyncha also possess endosymbionts that live within bacteriocytes and that are transmitted maternally. However, the phylogenetic position of the symbionts, mechanisms of vertical transmission, and the position and characteristics of bacteriocytes differ among major groups of insect hosts.

Codiversification

Phylogenetic analyses for *Buchnera* and aphids support strict cospeciation, with strictly vertical transmission over at least 100 million years (Fig. 3). This conclusion is based on analyses at several phylogenetic depths. First, phylogenies based on representatives from the superfamily Aphidoidea are completely concordant with phylogenies of their corresponding endosymbionts, based on deeply diverging lineages (Munson et al., 1991b; Moran et al., 1993). However, phylogenetic studies of such divergent groups might not reveal horizontal transfer if it has occurred between related aphid lineages, such as members of the same aphid tribe or genus. Phylogenetic concordance of *Buchnera* and 15 host species within the aphid genus *Uroleucon* was examined to determine if transfer among related host insects has taken place (Clark et al., 2000; Fig. 3). These aphids are restricted to plants within the Asteraceae, sometimes use the same host plant taxon, and often share natural enemies including parasitoid wasps that might act as vectors for symbiotic bacteria (Moran et al., 1998). Even these ecologically and phylogenetically related hosts showed strict codivergence with no horizontal transmission of *Buchnera* (Clark et al., 2000). Finally, molecular phylogenetic studies within a single aphid species, *Uroleucon ambrosiae*, indicate strict codivergence of symbiont genes and mitochondrial genes, indicating that even intraspecific transfer of *Buchnera* is absent (Funk et al., 2000). Since even rare instances of transfer would result in major discrepancies between phylogenies of host and symbiont, these combined results suggest that *Buchnera* has completely lost the capacity to live outside hosts and/or to invade new hosts.

Age of Endosymbioses in Sternorrhyncha

The shared ancestor of the superfamily Aphidoidea lived 100–200 million years ago (Heie, 1987). The observation of congruent phylogenies for aphids and *Buchnera* implies that the original infection is at least this old. Since the large majority of modern Sternorrhyncha have endosymbionts and feed on plant phloem sap, it seems likely that the common ancestor of the group also possessed endosymbionts. However, the current distribution of symbiont lineages at a higher taxonomic level, i.e., among families of Sternorrhyncha, indicates that there have been numerous independent infections, possibly involving replacements of ancestral symbionts with novel ones (Fig. 2). For example, some of the symbionts characterized for the Coccoidea, the sister group to Aphidoidea, are in the beta-Proteobacteria (Munson et al., 1992) although other groups of bacteria appear to be present in at least


host aphid gene phylogeny
Buchnera gene phylogeny
Aphididae
Pemphigus betae
Schlechtendalia chinensis
Melaphis rhois
Chaitophorus viminalis
Mindarus kinseyi
Uroleucon sonchi
Acyrthosiphon pisum
Macrosiphum rosae
Myzus persicae
Rhopalosiphum padi
Schizaphis graminum
Rhopalosiphum maidis
origin of symbiosis
ancestor of extant aphids 100-200 Mya
Uroleucon & relatives
Acyrthosiphon pisum
Macrosiphum rosae
Uroleucon erigeronense
Uroleucon caligatum
Uroleucon rurale
Uroleucon helianthicola
Uroleucon jaceicola
Uroleucon obscurum
Uroleucon rapunculoides
Uroleucon sonchi
Uroleucon solidaginis
Uroleucon jaceae
Uroleucon aeneum
Uroleucon rudbeckiae
Uroleucon astronomus
Uroleucon ambrosiae
colonization of Asteraceae <20 Mya

some coccoid species (Kantheti et al., 1996; Fukatsu & Nikoh, 2000). The Aleyrodoidea and the Psylloidea, sister clades within Sternorrhyncha, each contain a clade of gamma-Proteobacteria that are possibly sister clades to one another but not to *Buchnera* (Clark et al., 1993; Spaulding & von Dohlen, 1998).

Based on recent studies, phylogenetic relationships are congruent for the Psylloidea and their corresponding symbionts (Thao et al., 2000a). This result implies an ancient infection, since the Psylloidea are probably of similar age to the Aphidoidea. The infection may have predated divergence of Aleyrodoidea from Psylloidea; however, current information based on ribosomal sequences is not sufficient to fully resolve the sister group of psyllid symbionts.

Distinctive Characteristics of Endosymbionts and Coadaptation with Hosts

The 100 million years or more that *Buchnera* has resided in aphids implies more than a billion bacterial generations. How has it diverged from its nonsymbiotic ancestor? Extensive characterization of *Buchnera* from the aphid *Schizaphis graminum* (Rondani) combined with comparative studies of symbionts of some other aphid hosts indicate that *Buchnera* is closely related to *Escherichia coli* but shows a very divergent overall pattern of genome evolution (Baumann et al., 1999). The relationship to *E. coli* and other enteric bacteria has been verified repeatedly by sequence comparisons of homologous genes: almost all of the loci sequenced so far for *Buchnera* have clear homologs in the *E. coli* genome, in agreement with initial results based on 16S rDNA sequences (P. Baumann et al., 1999; Shigenobu et al., 2000).

One indicator of a distinctive pattern of genome evolution is evident from the extremely small genome size. The genome of *Buchnera* of *Acyrthosiphon pisum* is only about 640 kilobases and contains 590 genes (Charles & Ishikawa, 1999; Shigenobu et al., 2000), about a seventh of the size of the genome of *E. coli* at about 4500 kilobases and over 4000 genes. Similar sizes are found in *Buchnera* of other Aphidoidea (Wernegreen et al., 2000). An implication is that *Buchnera* underwent genome reduction, losing most of its DNA and most genes in the course of its evolution from a free-living ancestor. This resembles the situation in phylogenetically diverse intracellular pathogenic bacteria, which have repeatedly evolved small genomes (Andersson & Kurland, 1998; Moran & Wernegreen, 2000). Because bacterial genomes are mostly composed of functional genes, an implication is that *Buchnera* has lost most ancestral loci and corresponding functional capabilities.

A second distinctive aspect of genome evolution is revealed in the presence of plasmids bearing genes for biosynthesis of amino acids required by the host. Plasmid location of these genes is not typical of free-living bacteria, and the plasmid location allows increased gene copy number and overproduction of the gene product. *Buchnera* of some hosts, including members of the large family Aphididae, possess two plasmid types that are involved in production of tryptophan and leucine respectively (Lai et al., 1994; Bracho et al., 1995). On one of these, *trpEG*, the rate-limiting genes for tryptophan biosynthesis, are amplified as tandem repeats. For example, in the host *Schizaphis graminum*, each plasmid has four repeats and about four plasmids are present for each chromosomal copy, resulting in an effective 16-fold amplification. On the other plasmid, the four genes underlying leucine biosynthesis are present as single copies, along with replication genes and an unknown conserved open reading frame (van Ham et al., 1997). The plasmid is present in multiple copies, with an effective 20-fold amplification in *Buchnera* of *S. graminum* relative to genes on the main bacterial chromosome. The two types of plasmids are entirely different in organization, implying that they originated independently. Within each type, gene rearrangements and sequence evolution have occurred, resulting in differences among host taxa (Rouhbakhsh et al., 1996; van Ham et al., 1997; Silva et al., 1998; L. Baumann et al., 1999).

Coevolution and Plasmids in Aphid Symbionts

Both the leucine plasmid and the tryptophan plasmid are interpreted as adaptive modifications of the *Buchnera* genetic system that enable improved growth and reproduction of hosts. Bacterial plasmids are usually thought to be involved in gene transfer among bacterial strains or species. However, in the case of the *Buchnera* plasmids, phylogenetic evidence, based on sequences of genes

←

Figure 3. Phylogenetic congruence of aphids and their endosymbiotic bacteria, *Buchnera*, at the level of the family Aphididae and at the level of an ecologically homogeneous clade of aphid hosts, the genus *Uroleucon* and relatives. Trees are redrawn from Munson et al., 1991b; Moran et al., 1993; Moran et al., 1998; and Clark et al., 2000.

from both plasmid types, the bacterial chromosome, and the aphid mitochondrion, indicates strict vertical transmission of the plasmids within the clade corresponding to the aphid family Aphididae (Rouhbakhsh et al., 1997; Silva et al., 1998; L. Baumann et al., 1999), within the genus *Uroleucon* (Wernegreen & Moran, 2001), and within the species *Uroleucon ambrosiae* (Funk et al., 2000). In other words, not only is *Buchnera* itself entirely vertically transmitted, its plasmids also are, resulting in complete linkage of the plasmid-borne genes with genes on the bacterial chromosome and genes on the aphid mitochondrial chromosome. This situation might facilitate coadaptation of genes in different locations and improved coordination of expression of biosynthetic genes important to the fitness of aphid hosts.

Selfish Symbionts

Because *Buchnera* is strictly vertically transmitted, the possibilities for the establishment of symbiont traits that harm host fitness are quite limited. A bacterial mutant that replicated faster but provisioned hosts less well might spread within the population of bacteria in a single host and might then preferentially infect progeny. However, hosts bearing symbionts with "selfish" traits, i.e., those that benefit bacterial replication at the expense of host fitness, would experience negative selection within host populations even if bacteria are favorably selected within individual hosts. However, in small host populations that experience genetic drift, there is some potential for the spread of selfish traits despite counter selection at the host level. This is especially true if effects on hosts are small and if such alleles originate frequently through mutation (Michod, 1997; Rispe & Moran, 2000). Frequency of selfish mutations will depend, in part, on the size of the mutational target. Most mutations knocking out functionality of genes that underlie biosynthesis of host nutrients might have selfish effects, since they will compromise host nutrition but could improve rate of replication of the individual mutant cell lineage. The proportion of the genome underlying amino acid biosynthesis is substantial: based on the combined length of genes for synthesis of essential amino acids and on the full genome size (Charles & Ishikawa, 1999) this proportion could be as much as 10% or more. A high rate of origin of selfish mutations, combined with a short-term selective advantage within hosts, might result in a constant threat to aphid fitness.

Degenerative Symbionts?

Selection at the level of host individuals favors mutualistic, and functional, symbionts. However, such selection will be limited in effectiveness. First, since selection occurs mostly at the level of the animal host, the effective population size will be relatively small resulting in more genetic drift. This is because animal populations are relatively small (as compared to populations of most free-living bacteria). Furthermore, the symbionts forego any recombination with bacteria from other hosts, a conclusion strongly supported by phylogenetic results that support vertical transmission even for plasmid-borne genes are strictly vertically transmitted (Funk, Wernegreen & Moran, unpublished results).

A variety of evidence based on gene sequences suggests that *Buchnera* and other symbionts have been subject to the fixation of slightly deleterious mutations through genetic drift. First, rates of DNA sequence evolution are increased in symbionts, as expected if a larger proportion of mutations are fixed rather than eliminated through purifying selection (Moran, 1996; Spaulding & von Dohlen, 1998; Peek et al., 1998; Wernegreen & Moran, 1999). This acceleration is found at all loci analyzed and, in genes encoding polypeptides, is concentrated at sites causing amino acid substitutions, that is, sites likely to be subject to purifying selection (Moran, 1996; Wernegreen & Moran, 1999). The sequence changes lead to decreased stability in secondary structure of gene products (Lambert & Moran, 1998), as expected if they are slightly deleterious. These patterns appear to characterize many endosymbiont groups that are maternally transmitted in invertebrate hosts (Moran, 1996; Lambert & Moran, 1998; Peek et al., 1998). Another common feature of endosymbionts is the overproduction of the stress protein GroEL (Sato & Ishikawa, 1997; Aksoy, 1995). Bacterial GroEL functions to fold proteins that have lost their functional conformation (Ewalt et al., 1997), and it is typically overproduced in response to stress such as heat. Constitutively high levels in endosymbionts may function to maintain functionality of polypeptides that are less stable due to multiple amino acid substitutions resulting from mutation fixation through genetic drift (Moran, 1996). Finally, the small genome sizes of endosymbionts may be the consequence in part of genetic drift causing the fixation of mutations that eliminate function of genes that are beneficial but not essential. Once fixed, silenced alleles would disappear through neutral deletions.

Variable Host Nutrition and Variation in Symbiont Provisioning Capacity

Oddly, some *Buchnera* have undergone secondary loss of the extra copies of genes for biosynthesis of essential amino acids. Apparently, the ability to produce large amounts of these nutrients has been eliminated. In the case of *trpEG*, the genes for tryptophan production, some of the tandem repeats are eliminated as pseudogenes (gene copies with strong homology to functioning copies but with multiple frameshifts and stop codons in the coding region). In the case of the genes for synthesis of leucine, the plasmid copy number is reduced. An example of this situation is in *Buchnera* of *Diuraphis noxia*, an aphid that causes extensive damage to its *Triticum* host (wheat) and related grasses. In *D. noxia*, there are only one or two copies of plasmid *trpEG* and of *leuABCD* relative to each chromosomal gene; in contrast, other Aphididae have 16 to 24 copies for both sets of plasmid-encoded biosynthetic genes (Thao et al., 1998). Although this situation is unusual, it is not unique; other instances of reduced amplification level for amino acid biosynthetic genes are known in *Buchnera* of other aphid species (e.g., Baumann et al., 1997).

Are these reductions in gene copy number adaptive, potentially reflecting decreased host need for symbiont provisioning? Or are they maladaptive at the level of the host organism, potentially resulting from genetic drift or even selection at the intrahost level? Unusual enrichment of ingested phloem sap might lead to reduced dependence on symbiont biosynthesis and even selection to lower production levels by symbionts. In fact, studies on the nutrient composition of phloem sap ingested by *Diuraphis noxia* indicate that this species does ingest a diet enriched in essential amino acids. This enrichment is an effect of aphid feeding, which causes a characteristic damage to the infested plant and an increase of essential amino acids in the ingested phloem sap (Telang et al., 1999). The observed loss of gene copies may relate to this nutritional enrichment (Wernegreen & Moran, 2000). Nonetheless, haemolymph levels of leucine and tryptophan are unusually low in *D. noxia*, suggesting that these nutrients may be limiting to growth despite increased concentrations in ingested sap (Wernegreen & Moran, 2000). Like *D. noxia*, *Schizaphis graminum* causes damage on grass host plants that results in increases in amino acid concentrations in the diet. *Buchnera* of *S. graminum* do not show a loss of amplified gene copies, and *S. graminum* haemolymph is not low in tryptophan and leucine. Furthermore, *S. graminum* shows a much higher growth rate than does *D. noxia*. Thus, it appears that the decreased biosynthetic capability of *Buchnera* of *D. noxia* does correspond to lower tryptophan and leucine availability in *D. noxia*, and to a lower growth rate of the host, but whether this decrease is maladaptive for the host is not clear. Possibly, the low rate of reproduction and growth of *D. noxia* is part of a life history strategy that is connected with its habit of forming small sedentary colonies within curled leaves of hosts. Pseudogenes for *trpEG* are widespread among *D. noxia* populations and have arisen independently in other *Diuraphis* species that inflict similar damage on their host plants (Wernegreen & Moran, 2000).

Secondary Symbionts

Many species of Sternorrhyncha have more than one maternally transmitted symbiont, within the same individual insect. In aphids, symbionts other than *Buchnera*, called secondary or accessory symbionts, live outside the bacteriocytes, often in the nearby sheath cells (Buchner, 1965). Whether they are mutualistic or possibly pathogenic is not known. Associations are maternally transmitted but are evolutionarily less long-lived, with sporadic distribution among related species or even within a species (e.g., Chen & Purcell, 1998; Sandström et al., 2001). Some horizontal transmission also occurs, based on the presence of extremely closely related symbionts in phylogenetically divergent hosts (Chen & Purcell, 1998), but the mechanism of transfer is not known. Secondary symbionts also occur in psyllids (Fukatsu & Nikoh, 1998; Spaulding & von Dohlen, 1998) and in whiteflies (Clark et al., 1993). Secondary symbionts sometimes do not show the unusually fast sequence evolution and biased base composition that is typical of primary symbionts; their sequences may resemble those of characterized free-living bacteria such as the enterics (Chen & Purcell, 1998; Clark et al., 1993). However some secondary symbionts, perhaps those with longer histories as symbionts, do show some tendency toward accelerated evolution and biased base composition (Spaulding & von Dohlen, 1998; Fukatsu & Nikoh, 1998; Thao et al., 2000b).

Speculations on the Evolution of Endosymbioses

Endosymbioses may initially become established on the basis of nutritional benefits to hosts, explaining their concentration in animal clades, such as Sternorrhyncha, that live on restricted diets (Buchner, 1965). If infections benefit host fecundity, then infected maternal lineages will spread; similarly,

symbiont lineages with traits benefitting host fecundity will spread at the expense of symbiont lineages that lack these benefits. The latter is illustrated in *Buchnera* by the amplification of amino acid biosynthetic genes on plasmids: this feature apparently evolved few times and must have become fixed through selection at the level of host individuals. Subsequently, as symbionts become more integrated into the development and physiology of hosts, hosts may evolve dependencies on their bacteria that do not involve nutrition. This possibility, that hosts evolve increasing dependence on their long-term symbionts, is supported by repeated observations that aphids deprived of *Buchnera* cannot reproduce, even when limiting nutrients are supplied in artificial diets (Douglas, 1998). Although the association is beneficial and even obligate for hosts, the symbionts themselves may become less functional due to a large effect of genetic drift on their evolution, with the consequent fixation of mildly deleterious mutations.

The genome size of *Buchnera* is near the lower limit observed in bacteria (Maniloff, 1996; Charles & Ishikawa, 1999; Wernegreen et al., 2000). Most small genome bacteria studied so far are pathogens that form chronic associations with animal hosts. These pathogenic bacteria have lost many biosynthetic pathways, including almost all amino acid biosynthetic pathways, obtaining needed nutrients from host cells. In contrast, genetic characterization of *Buchnera* indicates that multiple amino acid biosynthetic pathways are retained (P. Baumann et al., 1999; Shigenobu et al., 2000). An implication is that *Buchnera* evolved from a free-living bacterium that became endosymbiotic and mutualistic as it lost genes: it did not evolve from an intracellar bacterium already possessing a reduced genome. The amino acid biosynthetic genes are too numerous and too dispersed in the genome to have been reacquired after being lost in a pathogenic ancestor. Thus, the idea that mutualistic bacteria arise from long-term pathogens can be discounted in this case and perhaps in others (Moran & Wernegreen, 2000).

Do other, independently acquired, symbionts of phloem-feeders show the same patterns discovered for *Buchnera*? The answer to this question will become evident during the next few years, through genome characterization of other symbionts. Initial results, for symbionts of psyllids, indicate some striking similarities (Spaulding & von Dohlen, 1998; Thao et al., 2000a).

Literature Cited

Aksoy, S. 1995. Molecular analysis of the endosymbionts of tsetse flies: 16S rDNA locus and over-expression of a chaperonin. Insect Molec. Biol. 4: 23–29.

Andersson, S. G. & C. Kurland. 1998. Reductive evolution of resident genomes. Trends Microbiol. 6: 263–268.

Bandi, C., M. Sironi, G. Damiani, L. Magrassi, C. A. Nalepa, U. Laudani & L. Sacchi. 1995. The establishment of intracellular symbiosis in an ancestor of cockroaches and termites. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 259: 293–299.

Baumann, L. & P. Baumann. 1994. Growth kinetics of the endosymbiont *Buchnera aphidicola* in the aphid *Schizaphis graminum*. Appl. Environm. Microbiol. 60: 3440–3443.

———, M. A. Clark, D. Rouhbakhsh, P. Baumann, N. A. Moran & D. J. Voegtlin. 1997. Endosymbionts (*Buchnera*) of the aphid *Uroleucon sonchi* contain plasmids with *trpEG* and remnants of *trpE* pseudogenes. Curr. Microbiol. 35: 18–21.

———, P. Baumann, N. A. Moran, J. Sandström & M. L. Thao. 1999. Genetic characterization of plasmids containing genes encoding enzymes of leucine biosynthesis in endosymbionts (*Buchnera*) of aphids. J. Molec. Evol. 48: 77–85.

Baumann, P., N. A. Moran & L. Baumann. 1999. Bacteriocyte-associated endosymbionts of insects. *In* M. Dworkin (editor), The Prokaryotes, 3rd Ed., A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. Springer-Verlag, New York. http://link.springer.de/link/service/books/10125/

Bracho, A. M., D. Martínez-Torres, A. Moya & A. Latorre. 1995. Discovery and molecular characterization of a plasmid localized in *Buchnera* sp., a bacterial endosymbiont of the aphid *Rhopalosiphum padi*. J. Molec. Evol. 41: 67–73.

Buchner, P. 1965. Endosymbiosis of Animals with Plant Microorganisms. Interscience Publishers, New York.

Charles, H. & H. Ishikawa. 1999. Physical and genetic map of the genome of *Buchnera*, the primary endosymbiont of the pea aphid *Acyrthosiphon pisum*. J. Molec. Evol. 48: 142–150.

Chen, D. Q. & A. H. Purcell. 1998. Occurrence and transmission of facultative endosymbionts in aphids. Curr. Microbiol. 34: 220–225.

Chen, X., S. Li & S. Aksoy. 1999. Concordant evolution of a symbiont with its host insect species: Molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. J. Molec. Evol. 48: 49–58.

Clark, M. A., L. Baumann, M. A. Munson, P. Baumann, B. C. Campbell, J. E. Duffus, L. S. Osborne & N. A. Moran. 1993. The eubacterial endosymbionts of whiteflies (Homoptera: Aleyrodoidea) constitute a lineage distinct from the endosymbionts of aphids and mealybugs. Curr. Microbiol. 25: 119–123.

———, N. A. Moran, P. Baumann & J. J. Wernegreen. 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. Evolution 54: 517–525.

Dohlen, C. D. von & N. A. Moran. 1995. Molecular phylogeny of the Homoptera: A paraphyletic taxon. J. Molec. Evol. 41: 211–223.

Douglas, A. E. 1998. Nutritional interactions in insect-microbial symbioses: Aphids and their symbiotic bacteria *Buchnera*. Annual Rev. Entomol. 43: 17–37.

Ewalt, K. L., J. P. Hendrick, W. A. Houry & F. U. Hartl. 1997. In vivo observation of polypeptide flux through the bacterial chaperonin system. Cell 90: 491–500.

Fukatsu, T. & N. Nikoh. 1998. Two intracellular symbiotic bacteria from the mulberry psyllid *Anomoneura mori* (Insecta, Homoptera). Appl. Environm. Microbiol. 64: 3599–3606.

Funk, D. J., L. Helbling, J. J. Wernegreen & N. A. Moran. 2000. Evolutionary congruence among multiple symbiont genomes in an aphid species. Under review, Proc. Roy. Soc. London, Ser. B, Biol. Sci. 267: 2517–2521.

Ham, R. C. H. J. van, A. Moya & A. Latorre. 1997. Putative evolutionary origin of plasmids carrying the genes involved in leucine biosynthesis in *Buchnera aphidicola*. J. Bacteriol. 179: 4768–4777.

Heie, O. E. 1987. Paleontology and phylogeny. Pp. 367–391 *in* A. K. Minks & P. Harrewijn (editors), Aphids: Their Biology, Natural Enemies and Control. World Crop Pests, Vol. 2A. Elsevier, Amsterdam.

Kantheti, P., K. S. Jayarama & H. S. Chandra. 1996. Developmental analysis of a female-specific 16S rRNA gene from mycetome-associated endosymbionts of a mealybug, *Planococcus lilacinus*. Insect Biochem. Molec. Biol. 26: 997–1009.

Lai, C.-Y., L. Baumann & P. Baumann. 1994. Amplification of *trpEG*: Adaptation of *Buchnera aphidicola* to an endosymbiotic association with aphids. Proc. Natl. Acad. Sci. U.S.A. 91: 3819–3823.

———, P. Baumann & N. A. Moran. 1996. The endosymbiont (*Buchnera* sp.) of the aphid *Diuraphis noxia* contains plasmids consisting of *trpEG* and tandem repeats of *trpEG* pseudogenes. Appl. Environm. Microbiol. 62: 332–339.

Lambert, J. L. & N. A. Moran. 1998. Deleterious mutations destabilize ribosomal RNA of endosymbionts. Proc. Natl. Acad. Sci. U.S.A. 95: 4458–4462.

Maniloff, J. 1996. The minimal cell genome: "On being the right size." Proc. Natl. Acad. Sci. U.S.A. 93: 10004–10006.

Michod, R. E. 1997. Cooperation and conflict in the evolution of individuality. I. Multi level selection of the organism. Amer. Naturalist 149: 607–645.

Moran, N. A. 1996. Accelerated evolution and Muller's ratchet in endosymbiotic bacteria. Proc. Natl. Acad. Sci. U.S.A. 93: 2873–2878.

——— & A. Telang. 1998. The evolution of bacteriocyte-associated endosymbionts in insects. Bioscience 48: 295–304.

——— & J. J. Wernegreen. 2000. Are mutualism and parasitism irreversible evolutionary alternatives for endosymbiotic bacteria? Insights from molecular phylogenetics and genomics. Trends Ecol. Evol. 15: 321–326.

———, M. A. Munson, P. Baumann & H. Ishikawa. 1993. A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 253: 167–171.

———, M. A. Kaplan, M. Gelsey, T. Murphy & E. Scholes. 1998. Phylogenetics and evolution of the aphid genus *Uroleucon* based on mitochondrial and nuclear DNA sequences. Syst. Entomol. 24: 85–93.

Munson, M. A., P. Baumann & M. G. Kinsey. 1991a. *Buchnera* gen. nov. and *Buchnera aphidicola* sp. nov., a taxon consisting of the mycetocyte-associated, primary endosymbionts of aphids. Int. J. Syst. Bacteriol. 41: 566–568.

———, ———, M. A. Clark, L. Baumann, N. A. Moran, D. J. Voegtlin & B. C. Campbell. 1991b. Aphid-eubacterial endosymbiosis: Evidence for its establishment in an ancestor of four aphid families. J. Bacteriol. 173: 6321–6324.

———, ——— & N. Moran. 1992. Phylogenetic relationships of mealybug endosymbionts (Homoptera: Pseudococcidae) based on 16S rDNA sequences. Molec. Phylogenet. Evol. 1: 26–30.

Peek, A. S., R. A. Feldman, R. A. Lutz & R. C. Vrijenhoek. 1998. Cospeciation of chemoautotrophic bacteria and deep sea clams. Proc. Natl. Acad. Sci. U.S.A. 95: 9962–9966.

Rispe, C. & N. A. Moran. 2000. Accumulation of deleterious mutations in endosymbionts: Muller's ratchet with two levels of selection. Amer. Naturalist 156: 425–441.

Rouhbakhsh, D., C.-Y. Lai, C. D. von Dohlen, L. Baumann, P. Baumann, N. Moran & D. J. Voegtlin. 1996. The tryptophan biosynthetic pathway of aphid endosymbionts (*Buchnera*): Genetics and evolution of plasmid-associated *trpEG* within the Aphididae. J. Molec. Evol. 42: 414–421.

Sandström, J. & N. Moran. 1999. How nutritionally imbalanced is phloem sap for aphids? Entomol. Exp. Appl. 91: 203–210.

———, J. A. Russell, J. P. White & N. A. Moran. 2001. Independent origins and horizontal transfer of bacterial symbionts of aphids. Molec. Ecol. 10: 217–228.

Sato, S. & H. Ishikawa. 1997. Expression and control of an operon from an intracellular symbiont which is homologous to the groE operon. J. Bacteriol. 179: 2300–2304.

Schröder, D., H. Deppisch, M. Obermeyer, G. Krohne, E. Stackebrandt, B. Hölldobler, W. Goebel & R. Gross. 1996. Intracellular endosymbiotic bacteria of *Camponotus* species (carpenter ants): Systematics, evolution and ultrastructural characterization. Molec. Microbiol. 21: 479–490.

Shigenobu, S., H. Watanabe, M. Hattori, Y. Sakaki & H. Ishikawa. 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. Nature 407: 81–86.

Silva, F., R. C. H. J. van Ham, B. Sabater & A. Latorre. 1998. Structure and evolution of leucine plasmids carried by the endosymbiont (*Buchnera aphidicola*) from aphids of the family Aphididae. F.E.M.S. Microbiol. Lett. 168: 43–49.

Spaulding, A. W. & C. D. von Dohlen. 1998. Phylogenetic characterization and molecular evolution of bacterial endosymbionts in psyllids (Hemiptera: Sternorrhyncha). Molec. Biol. Evol. 15: 1506–1513.

Telang, A., J. Sandström, E. Dyreson & N. A. Moran. 1999. Feeding damage by *Diuraphis noxia* results in a nutritionally enhanced phloem diet. Entomol. Exp. Appl. 91: 403–412.

Thao, M. L., L. Baumann, P. Baumann & N. A. Moran. 1998. Endosymbionts (*Buchnera*) from the aphids *Schizaphis graminum* and *Diuraphis noxia* have different copy numbers of the plasmid containing the leucine biosynthetic genes. Curr. Microbiol. 36: 238–240.

———, N. A. Moran, P. Abbot, E. B. Brennan, D. H. Burckhardt & P. Baumann. 2000a. Cospeciation of psyllids and their prokaryotic endosymbionts. Appl. Environm. Microbiol. 66: 2898–2905.

———, M. Clark, L. Baumann, E. B. Brennan, N. A. Moran & P. Baumann. 2000b. Secondary symbionts of psyllids have been acquired multiple times. Curr. Microbiol. 41: 300–304.

Wernegreen, J. J. & N. A. Moran. 1999. Evidence for genetic drift in endosymbionts (*Buchnera*): Analyses of protein-coding sequences. Molec. Biol. Evol. 16: 83–97.

——— & ———. 2000. Decay of mutualistic potential in aphid endosymbionts through silencing of biosynthetic loci: *Buchnera* of *Diuraphis*. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 267: 1423–1431.

——— & ———. 2001. Evolution of a bacterial-plasmid association with strict vertical transmission: Biosynthetic plasmids of aphid symbionts. J. Bacteriol. 183: 785–790.

———, H. Ochman, I. Jones & N. A. Moran. 2000. The decoupling of genome size and sequence divergence in a symbiotic bacterium. J. Bacteriol. 182: 3867–3869.

CHEMICAL MEDIATION OF COEVOLUTION: PHYLOGENETIC EVIDENCE FOR APIACEAE AND ASSOCIATES[1]

May R. Berenbaum[2]

ABSTRACT

The chemical diversity of the Apiaceae has made the family of particular significance in discussions of plant/insect interactions for close to a century. As forbs with distinctive chemistry and a specialized insect fauna, species in the Apiaceae and their associated herbivores represent an ideal system for examining competing hypotheses accounting for hostplant acquisition patterns. Comparisons of the phylogenetic history of colonization of Apiaceae across a number of taxa reveal that host shifts to the family originate in only a narrow range of taxa; chemical similarities among those taxa, rather than geographic proximity or phylogenetic relationships, are the most plausible basis for the observed host shifts. Similar ecologies among species in this narrow range of taxa, including similar herbivore faunas, may well account for similarities in overall chemical profiles. The patterns documented here are consistent with reciprocal coevolutionary interactions between herbivorous insects and their hostplants.

Key words: *Agonopterix*, Apiaceae, coevolution, *Depressaria*, *Euleia*, furanocoumarin, *Greya*, insect-plant interaction, *Oreina*, *Papaipema*, *Papilio*.

The family Apiaceae contains over 3000 species worldwide; these can be found in a wide range of habitats, including deserts, marshes, basaltic bluffs, forests, subalpine tundra, woodlands, and wasteplaces (Berenbaum, 1990). Morphologically, the Apiaceae are a fairly conservative family, one of the few so-called "natural plant families" of Theophrastus of Eresus (the "father of botany"). Theophrastus recognized the taxonomic significance of the "naked seeds" that characterize some members of the group (the pair of one-seeded mericarps that constitute a schizocarp), as well as the distinctive umbelliform inflorescence. These plants were classified as *Narthekodes*, from the Greek *Narthex*, meaning "plant." In Latin, the group acquired the name *Ferulacea*; the term "Umbelliferae" did not appear until the late sixteenth century, with Daleschamps's 1586 *Historia generalis plantarum* as an example (Constance, 1971). To the Apiaceae goes the distinction of being the first plant family to be monographed, when Robert Morison published his *Plantarum umbelliferarum distributio nova* in 1672 (Hedge, 1973; Downie et al., 1998).

In striking contrast with their morphological conservatism, the Apiaceae display considerable diversity in chemistry. The earliest manifestations of these chemical differences appear in the myriad ways in which apiaceous plants have been used historically by cultures all over the world. Everywhere they grow, they have been utilized throughout the ages by people for all manner of purposes. In his *Greek Herbal,* Dioscorides listed close to 50 different Apiaceae among his useful plants, most of which were classified as "herbs." The aromatic nature of many apiaceous plants, attributable to essential oils in glands on seeds and elsewhere, makes them well suited for this function. Dill (*Anethum graveolens* L.), coriander (*Coriandrum sativum* L.), fennel (*Foeniculum vulgare* Mill.), cumin (*Cuminum cyminum* L.), and anise (*Pimpinella anisum* L.) have filled this role more or less continuously since that time. Petrified caraway seeds (*Carum carvi* L.) were excavated from prehistoric Lake Dwellings in Switzerland (Rohde, 1922). As well, many members with tuberous roots or analogous structures have found use as staple foods—carrots (*Daucus carota* L.), parsnip (*Pastinaca sativa* L.), and celery (*Apium graveolens* L.) among them. Although, as French (1971: 387) pointed out, "no known civilization has been based on the cultivation of parsnips or carrots," in North America,

[1] I thank Stephen Downie for sharing reprints and insights, Arthur Zangerl and Terry Harrison for helpful comments on the manuscript, and I especially thank Victoria Hollowell and her colleagues at *Annals of the Missouri Botanical Garden* for patience well beyond reasonable expectations. This work was funded in part by NSF 99 03867 and IBN 96-30442.

[2] Department of Entomology, 320 Morrill Hall, University of Illinois, 505 S. Goodwin, Urbana, Illinois 61801-3795, U.S.A.

the Nez Perce used the swollen roots of *Perideridia* and *Lomatium* species as a staple food source.

In large part due to their diverse chemistries, apiaceous plants have found ubiquitous use for a panoply of disorders and diseases, including, among others, gastrointestinal disturbances, respiratory diseases, urogenital problems (including syphilis), and skin problems (wounds, bites, stings, boils, rashes, and warts). A few have found use as aphrodisiacs. According to Dioscorides, "Parsley—especially Bastard or Wild Parsley—provokes venery and bodily lust & erection of the parts" (Rohde, 1922). Carrots were used to treat jaundice in Europe, most likely because of their orange color, in an apparent application of the doctrine of signatures (French, 1971). Rycharde Bankes's 1525 *Herball*, possibly the first herbal printed in Britain, devoted quite a bit of text to apiaceous plants. Caraway was recommended "to destroy wycked wynds and y coughe; it is good for y frenesy & for bytynge of venemous beestes; for scabbes & teters." He also claimed the oil "restoreth heere as it is fallen awaye." As for celery, according to Bankes, "The virtue of this Herbe is thus: it wyll make a man to pysse and open y stoppynge of y lyver." The seed "ground well with Seed of Rue/ pepper & salt/ then mixt with Wyne & drunke/ was goode for the colde & wycked humoures in y stomake/lyver or longes." With respect to chervil (*Anthriscus cerefolium* (L.) Hoffmann), "the vertue of this herbe is thus: it shall make a man to pysse well . . . if it be dronken with Wyne it delyuereth a man of ache in the raynes [kidneys] & letteth out and unbyndeth y wycked wyndes." John Gerard was perhaps the most well known of English herbalists—*The Herball or generall historie of plantes* was published in 1597. According to Gerard, "Caruwaies consume winde; are delightful to the stomacke and taste and provoke urine." In 1649, Nicholas Culpepper produced his *Physicall Directory (an Astrologo-Phisical Discourse of the Vulgar Herbs of this Nation: Containing a Compleat Method of Physick, whereby a man may preserve his both in Health; or Cure himself, being Sick, for three pence Charge, with such things only as grow in England, they being most fit for English Bodies* (1661 edition) (Arber, 1938). In this work, angelica (*Angelica archangelica* L.) was recommended as a tonic, stimulant, carminative, expectorant, and diaphoretic, as well as a remedy for warding off devils and witches.

Chemical potency underlies the historical use of Apiaceae for not-so-therapeutic purposes as well. In ancient Greece, one of the plants used for the execution of criminals, *Conium maculatum* L. (poison hemlock), was used for dispatching Socrates for corrupting the morals of Athenian youth (Fairbairn, 1971). In imperial Rome, plant poisoning was an important factor in determining patterns of primogeniture and succession. The practice of plant poisoning probably reached its peak in medieval Europe, and in the courts of Italy and France apiaceous species were routinely included in the poisoner's arsenal. The twelfth century herbal of Apuleius, for example, specifically makes note of the poisonous properties of water dropwort, *Oenanthe crocata* L. (which was employed as a fish poison when it was not being used for more insidious purposes).

Chemical Diversity Documented

The ethnobotanical importance of the Apiaceae, specifically of Apiaceae subfamily Apioideae, is a reflection of the remarkable chemical diversity within the family; these plants are distinctive in the diversity, abundance, and uniqueness of their chemistry. Throughout the nineteenth and twentieth centuries, as the scientific study of analytical chemistry and then phytochemistry grew more sophisticated, an impressive inventory was documented. Apiaceae, for example, are well endowed with essential oil constituents. Although they share monoterpenes with a large number of taxa, they are distinctive in producing certain essential oil constituents and non-volatile biosynthetically related compounds. The characteristic terpenoids of Apiaceae include esters of monoterpene aldehydes, daucan-type sesquiterpenes, ligustilide-type compounds, and guaianolide and germacranolide sesquiterpene lactones (Hegnauer, 1971).

Also present as essential oil constituents are phenylpropanoid derivatives. There are at least five different groups of phenylpropenes, based on patterns of hydroxylation of the benzene ring (Harborne, 1971). One widespread representative of 3,4,5-trihydroxy-substituted phenylpropenes is myristicin, containing a methylenedioxyphenyl (MDP) ring; this compound is reported to occur in 14 genera within the family. Isoeugenol epoxide esters are found in Apiaceae, but they are extremely rare outside the family. Also unusual among plants are the 2-methylchromones that characterize Apiaceae.

Other phenylpropanoid derivatives include the coumarins; the Apiaceae produce these compounds in abundance and diversity rivaled by few other families (Berenbaum, 1991). Among the distinctive coumarin derivatives are both linear and angular furanocoumarins; pyranocoumarins are also frequently found. Although hydroxycoumarins are

widespread among plants, occurring in over 30 families, the presence of isopentenyl-substituted coumarins in abundance further distinguishes the Apiaceae from other families.

Polyacetylenes are yet another hallmark of the family, occurring in all three subfamilies (Bohlmann, 1971). Whereas C-17 polyacetylenes (such as falcarinol) are most frequently encountered, C-13 and C-15 polyacetylenes are also known from a variety of taxa.

Alkaloid production is extremely limited within the Apiaceae, being more or less restricted to the genus *Conium*. The structural type represented within this genus is relatively unusual. Coniine and related piperidine alkaloids are narrowly distributed throughout the plant kingdom and are best known from this genus. The deadly action of poison hemlock is attributable to the presence of these alkaloids, which function as potent neurotoxins to vertebrates (Fairbairn, 1971).

Finally, even storage compounds are chemically distinctive within the Apiaceae (Hegnauer, 1971). Seeds generally contain large quantities of fatty oils; virtually universal in occurrence throughout the family is the fatty acid petroselinic acid, rarely found elsewhere. As for carbohydrates, the raffinose isomer umbelliferose is a family character.

Ecological Significance of Apiaceae Chemistry

The distinctive nature of the chemistry of Apiaceae typified a pattern that had been revealed by the last quarter of the nineteenth century after inspection of a broad range of plants. Perhaps most distinctive was the fact that there were certain types of chemicals possessed to some degree by all plants—chlorophyll, for one, used as a photosynthetic pigment, or the amino acids that make up plant proteins. Others were exceedingly idiosyncratic in their distribution—restricted to only a single family, genus, or even species of plant, and varying with season, climate, soil type, and even age of the individual plant. It was reasoned that such compounds were unlikely to be essential to the day-to-day physiological needs of a plant and thus they were called secondary compounds. The Apiaceae proved to be an excellent source of these secondary compounds.

If these compounds play no role in primary physiological processes, the question then arose as to what their function really is in the plant. By the mid-nineteenth century, a hypothesis was put forward. In a study of the relationships between snails and plants, Stahl (1888—quoted in Fraenkel, 1959: 1466) remarked:

"We have long been accustomed to comprehend many manifestations of the morphology [of plants] . . . as being due to the relations between plants and animals, and nobody, in our special case here, will doubt that the external mechanical means of protection of plants were acquired in their struggle. . . with the animal world . . . . In the same sense, the great differences in the nature of chemical products [Excrete] and consequently of metabolic processes, are brought nearer to our understanding, if we regard these compounds as means of protection, acquired in the struggle with the animal world. Thus, the animal world which surrounds the plants deeply influenced not only their morphology, but also their chemistry."

Secondary compounds, then, were suggested to play an important ecological function to the plant—by mediating its interactions with other organisms. The Apiaceae have been a key focus for testing this hypothesis over the years. Brues (1920: 322), writing on associations between plants and insects, remarked that the family is infrequently colonized by insects due to its diverse and conspicuous chemistry (e.g., "We may readily see that the generally strong-scented Labiatae and Umbelliferae and the milky Euphorbiaceae might require great adaptation on the part of larvae eating them"). It did not escape his notice that the insects associated with these plants are highly specialized. Dethier (1941) traced patterns of chemical similarity among hosts of *Papilio* swallowtails, including Apiaceae, and suggested that chemical similarities among plants, particularly in essential oil constituents, may facilitate colonization of novel hostplants.

Fraenkel (1959) resurrected Stahl's notion of chemical mediation of ecological interactions and bolstered it with the accumulated empirical evidence of the intervening seven decades that ecological interactions were in fact the raison d'être of plant secondary compounds. The Apiaceae were one of six families of plants and their associates provided as examples of how chemical similarities underlie patterns of hostplant use by specialized herbivores. Moreover, he provided an evolutionary mechanism to account for the idiosyncratic distribution of plant secondary chemicals—what he called reciprocal adaptive evolution.

Five years later, Ehrlich and Raven (1964) dubbed this reciprocal process coevolution and proposed a multi-step scenario to account for diversification of secondary metabolism—

1. by mutation or recombination, plants acquire a novel chemical compound; by chance, this com-

pound reduces the suitability of the plant as food for herbivores;

2. protected from herbivory, the plant can enter a new adaptive zone and diversification can take place;

3. by mutation or recombination, a phytophagous insect acquires the means to detoxify or otherwise tolerate the erstwhile toxin and thus can utilize the previously protected plant;

4. this insect then enters a new adaptive zone and can undergo evolutionary diversification;

5. the erstwhile toxin can become a host recognition cue or attractant for its adapted herbivores.

Again, Apiaceae and their insect associates provided fuel for the argument. In particular, these authors remarked on the ability of species in the genus *Papilio* to feed freely on Rutaceae and Apiaceae, two chemically similar families; yet despite the close taxonomic relationship between Apiaceae and Araliaceae, no papilionid butterflies were known at the time to feed on species in the latter family.

With their multistep scenario, Ehrlich and Raven suggested that coevolution between insects and plants was responsible at least in part for the tremendous diversification of these two groups and hence for the diversity observed in most terrestrial ecosystems. Thus, this coevolutionary process, subsequently labeled the "escape and radiate" model (Thompson, 1999), accounts not only for the biochemical diversity of angiosperm plants and herbivorous insects, but also for the tremendous diversification of plant secondary compounds.

Ehrlich and Raven's (1964) paper inspired decades of both theoretical and empirical work designed to document all or part of the evolutionary scenario. Berenbaum (1983) combined literature and original data to apply the stepwise scenario to furanocoumarins, a group of apiaceous secondary chemicals. Furanocoumarins are typical secondary compounds in that they are idiosyncratically distributed among plants. They have been reported from only a handful of angiosperm families, and they are ubiquitous and diverse in only two families—Rutaceae and Apiaceae. Within the Apiaceae, they are common only in the subfamily Apioideae. There are two classes of furanocoumarins—linear, with the furan ring attached in line with the coumarin nucleus, and angular, with the furan ring at the 7,8 position and hence at an angle. The citrus and carrot families have been long known to physicians as having phytophotodermatitic properties. Casual contact with the aboveground parts results in the eruption of painful blisters, followed in severe cases by permanent scarring and elevated pigmentation of the skin. The agents responsible for these conditions are the furanocoumarins (Berenbaum, 1991). They are relatively unusual among plant compounds in that, in the presence of ultraviolet light, they are able to cross-link strands of DNA and hence prevent transcription. Thus, they are toxic to a wide range of organisms, including bacteria, fungi, and some viruses; plants containing furanocoumarins cause problems to livestock including ducks, geese, cattle, horses, and pigs; and they are, of course, toxic to insects.

All known furanocoumarins are thought to derive from a single biosynthetic precursor, umbelliferone (7-hydroxycoumarin). Umbelliferone and related hydroxycoumarins, occurring in approximately three dozen plant families, are far more widespread among plant families than are the derivative furanocoumarins. Among the furanocoumarins the two principal structural classes derive from the activity of two different location-specific prenylating enzymes: linear, with the prenyl group ring attached at the 6 position, and angular, with the prenyl group attached at the 8 position. Linear furanocoumarins, known from about a dozen plant families, are more widely distributed in turn than are the angular furanocoumarins, which are restricted to approximately three plant families. When tested against a generalist insect, linear furanocoumarins are appreciably more toxic than are hydroxycoumarins, and, when tested against an adapted specialist, angular furanocoumarins are more toxic than are linear furanocoumarins. This progressive increase in toxicity, corresponding to biosynthetic advancement, is consistent with the Ehrlich and Raven scenario. Moreover, as Ehrlich and Raven predicted, plant taxa with more advanced coumarins are more species-rich than are taxa with less advanced coumarins, and insect taxa feeding on plants with higher coumarins are more species-rich than are taxa feeding on plants with simple coumarins (Table 1). This increase in diversity was interpreted as evidence of the existence of Ehrlich and Raven's "adaptive zones." Similarly, lepidopteran groups associated with hostplants with advanced coumarins (such as furanocoumarins) are more species-rich than close relatives that are associated with plants containing only simple coumarins (Table 1), again suggesting entry into a new adaptive zone with subsequent diversification.

The suggestion that the evolution of biochemical innovations in plants and insects initiates adaptive radiation led Mitter and Brooks (1984) to predict that coevolution between plants and animals should lead to parallel cladogenesis—essentially, that these alternating periods of diversification in con-

Table 1. Putative adaptive radiations associated with biochemical novelties in Apiaceae-insect associations (LFC = linear furanocoumarin, AFC = angular furanocoumarin, HC = hydroxycoumarin). (Berenbaum, 1983.)

A. Chemical diversity in genera within Apiaceae

Coumarin chemistry	Avg. no. spp./genus
Without furanocoumarins or with dihydroxyfuranocoumarins only	12.1 ± 3.6
With linear furanocoumarins only	17.4 ± 5.8
With linear and angular furanocoumarins	67.3 ± 16.8

B. Species diversity within the genus *Papilio* according to hostplant chemistry

Papilio section	Hostplant chemistry	No. spp.
Section II	LFC, AFC, HC	139
Section IV	LFC, HC	34
Section V	HC	18
Section I	HC	9
Section III	HC	8

C. Species diversity within the Depressariinae according to hostplant chemistry

Depressariine genus	Hostplant chemistry	No. spp.
Depressaria	LFC, AFC, HC	100
Agonopterix	LFC, AFC, HC	125
Exaeretia	HC	38
Apachea	LFC, HC	1
Bibarrambla	HC	1
Nites	NC	5

cert will be reflected in the evolutionary record as phylogenies with identical topologies. This suggestion stimulated a wave of effort among systematists to compare cladograms of herbivorous insects and their associated hostplants. There have been some striking examples of congruent cladogenesis, e.g., *Tetraopes* and their milkweed hosts (Farrell & Mitter, 1998), but the majority of tests have failed to document such patterns. At the same time, studies of other groups have revealed patterns that are entirely inconsistent with parallel cladogenesis and are more consistent with models of sequential evolution, whereby insects colonize plants long after taxonomic and chemical diversity have evolved, rather than coevolution. Menken (1996) examined relationships among nine western European *Yponomeuta* moth species and determined that, within this species-group, four hostplant families representing three orders are utilized. Even for species groups utilizing a single hostplant family, phylogenetic relationships among insect herbivores do not necessarily reflect tribal relationships among hostplants (Funk et al., 1995).

The fact that patterns other than parallel cladogenesis have been demonstrated, with perhaps greater frequency, has been interpreted by some (Jermy, 1993; Schoonhoven et al., 1998) as a justification for rejecting coevolution as a mechanism for species interaction. Cited in support of this rejection is literature suggesting that insects rarely act as selective agents on their hostplants—a necessary prerequisite to reciprocal selection. Many still maintain this position (Schoonhoven et al., 1998), despite abundant evidence that under certain circumstances insect herbivores can indeed exert selection on their hostplants (Marquis, 1992). Another factor leading to skepticism about coevolution is the temporal incongruence of taxa; in many cases, it is clear that plant hosts diversified long before insect colonization took place (Futuyma, 1983; Funk et al., 1995). Thompson (1999) argued, however, that the Ehrlich and Raven (1964) scenario does not in fact predict parallel cladogenesis at all; rather, the phylogenetic pattern that is generated by stepwise reciprocal evolution is one of alternating "starburst" episodes of diversification. Thus, the infrequency of congruent cladogenesis does not in itself lead to a rejection of coevolution.

A more reasonable view of interaction between plants and insect herbivores is that coevolution is not a universal phenomenon characterizing all forms of interactions between herbivorous insects and their hostplants, but that it successfully describes some forms of interactions at particular taxonomic levels under certain ecological circumstances. Indeed, given the staggering abundance of plant-feeding insects and the plants they consume, the expectation of a single model of any sort seems unrealistic at best. Moreover, the intense focus on insect and plant systematics and phylogeny has led many investigators to overlook the putative chemical mediators of the process. Tests of chemical facilitation of hostplant shifts and subsequent diversification are few and far between.

Janz and Nylin (1998) conducted a study that sheds some light on the nature of sequential colonization. Appropriately, these authors reexamined the groups that had originally inspired Ehrlich and Raven (1964) to propose their coevolutionary scenario. In this study, a butterfly phylogeny was assembled from a broad array of studies and was compared with a plant phylogeny derived from molecular analyses (Chase et al., 1993). From this study, the authors concluded that host shifts do tend to occur between closely related plants, with

Table 2. Specialist insects in North America exclusively or almost exclusively associated with Apiaceae (Berenbaum, 1990).

	Genus	Number of specialists/ number in genus	Feeding habits[a]
Homoptera			
Aphididae	*Aphis*	5/153	PS
	Cavariella	4/11	PS
	Hyadaphis	1/2	PS
Hemiptera			
Miridae	*Orthops*	1/11	PS
Diptera			
Psilidae	*Psila*	1/1	RM
Agromyzidae	*Phytomyza*	14/	LM
Tephritidae	*Euleia*	1/2	LM
Coleoptera			
Curculionidae	*Apion* (*Fallapion*)	4/10 (125 total)	SM
	Smicronyx	2/69	SM
	Listronotus	1/28	RM
Lepidoptera			
Prodoxidae	*Greya*	3/15	SM
Oecophoridae	*Agonopterix*	7/34	LF
	Depressaria	18/21	LF
Epermeniidae	*Epermenia*	3/3	SM, LM
Noctuidae	*Papaipema*	2/22	SB
Papilionidae	*Papilio*	11/26	LF

[a] PS = phloem sucker, RM = root miner, LM = leaf miner, SM = seed miner, LF = leaf and flower chewer, SB = stem borer.

colonization of more distantly related plants more common in tree-feeding taxa. They suggested that a possible mechanism underlying the facility with which tree-feeding taxa colonize new hosts is the reduced degree of chemical differentiation among trees, relative to forbs. Thus, for tree-feeding species, the expectation of coevolutionary interactions—and hence parallel cladogenesis—should be low; correspondingly, for forb-feeding species, the expectation should be higher.

Acquisition of Apiaceae Hostplants—Phylogeny or Chemistry?

As forbs with a specialized insect fauna and diverse chemistry, the Apiaceae and their associated herbivores (Table 2) represent an ideal system for examining competing hypotheses accounting for hostplant acquisition. The principal insect associates in North America are in the order Lepidoptera, notably in the family Papilionidae; within the genus *Papilio,* a group of approximately six species within the *machaon* complex are associated with Apiaceae. Also within the Lepidoptera is the family Oecophoridae (in part; now subfamily Depressariinae in Elachistidae); two genera, *Depressaria* and *Agonopterix,* are noteworthy in the extent to which species utilize apiaceous hostplants. Within the genus *Papaipema* (Noctuidae), Apiaceae-feeding is also well documented, as it is for a handful of microlepidopteran species in the Prodoxidae and Epermeniidae.

In Diptera, there are representatives of two families associated with Apiaceae; within the family Agromyzidae, several species of *Phytomyza* are specialists, forming distinctive linear leaf mines in the foliage of their hosts. Also mining the leaves of apiaceous hostplants are species of Tephritidae in the genus *Euleia.*

Within other orders, feeding on Apiaceae is infrequently encountered (Burki & Nentwig, 1997). Among Coleoptera, specialization on Apiaceae is found in at least two families: Curculionidae (*Apion* and, in Europe, *Liophloeus*) and Chrysomelidae (in Europe, *Oreina*). There are relatively few hemimetabolous insects specialized on Apiaceae—among them are *Aphis/Cavariella/Anuraphis* (Aphididae) and, in Europe, *Eupteryx aurata* (Cicadellidae) in Homoptera, and *Orthop*s in Hemiptera. In part due

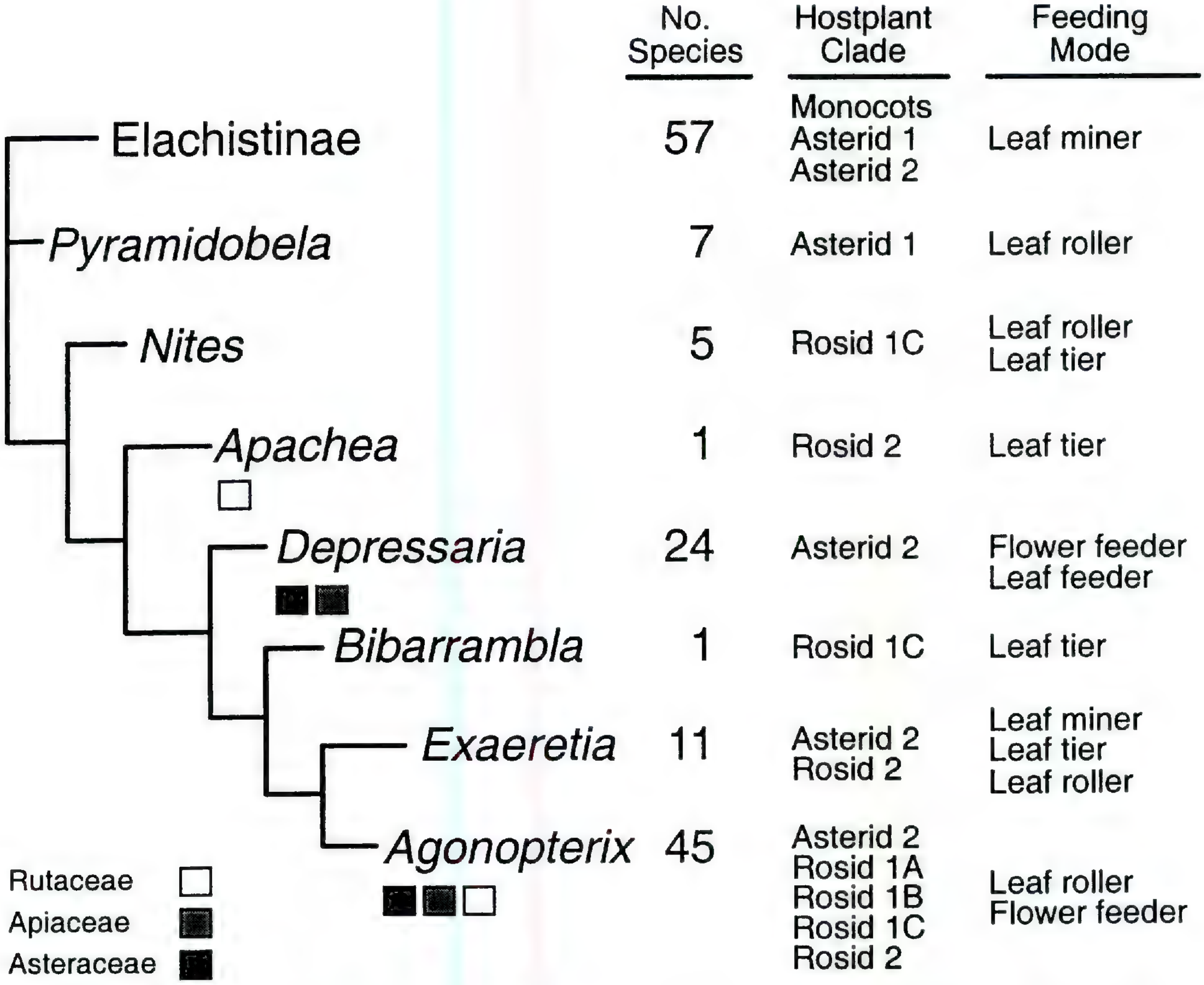


Figure 1. Phylogenetic relationships and hostplant associations in depressariine Elachistidae (modified from Berenbaum & Passoa, 1999; plant clades as in Chase et al., 1993).

to the economic importance of the Apiaceae, and in part due to general recognition of the importance of the family as a paradigm for the study of coevolution, a remarkable number of phylogenetic studies have been conducted to date on the herbivores associated with the family.

Depressariines

Berenbaum and Passoa (1999) recently completed an analysis of phylogenetic relationships among the depressariine Oecophoridae (= Elachistidae) (Fig. 1). The Depressariinae can be defined as a monophyletic insect group based on the tubular mesothoracic spiracle, a character first noted by Nakamura (1981). In the Depressariinae, *Agonopterix* and *Depressaria*, the only two Apiaceae-feeding Nearctic depressariines, are both monophyletic. Although *Depressaria* is defined by a homoplasious apomorphy, at least the two major North American Apiaceae-feeding species groups within the genus (*pastinacella* and *douglasella*) are defined by autapomorphies in their male genitalia and larval pinacula (S. Passoa & M. Berenbaum, unpublished data). A phylogeny for *Agonopterix* is not presently available.

The existence of monophyletic groups is important because within the context of coevolution adaptive radiations occur due to the acquisition of key innovations associated with overcoming plant defenses (Ehrlich & Raven, 1964). Farrell et al. (1992) suggested that sister-group comparisons could be used to determine whether an adaptive radiation has taken place; because sister groups are equal in age, a significantly greater number of species in one branch could be construed as evidence of evolutionary success. *Agonopterix*, with 42 species, is more diverse than its sister group *Exaeretia*, with 10 species, at least in North America (Hodges et al., 1983). On a generic level, *Depressaria*, with 24 species, is less diverse than its sister group, composed of *Bibarrambla* (1 species), *Exaeretia* (10 species), and *Agonopterix* (43 species). In contrast,

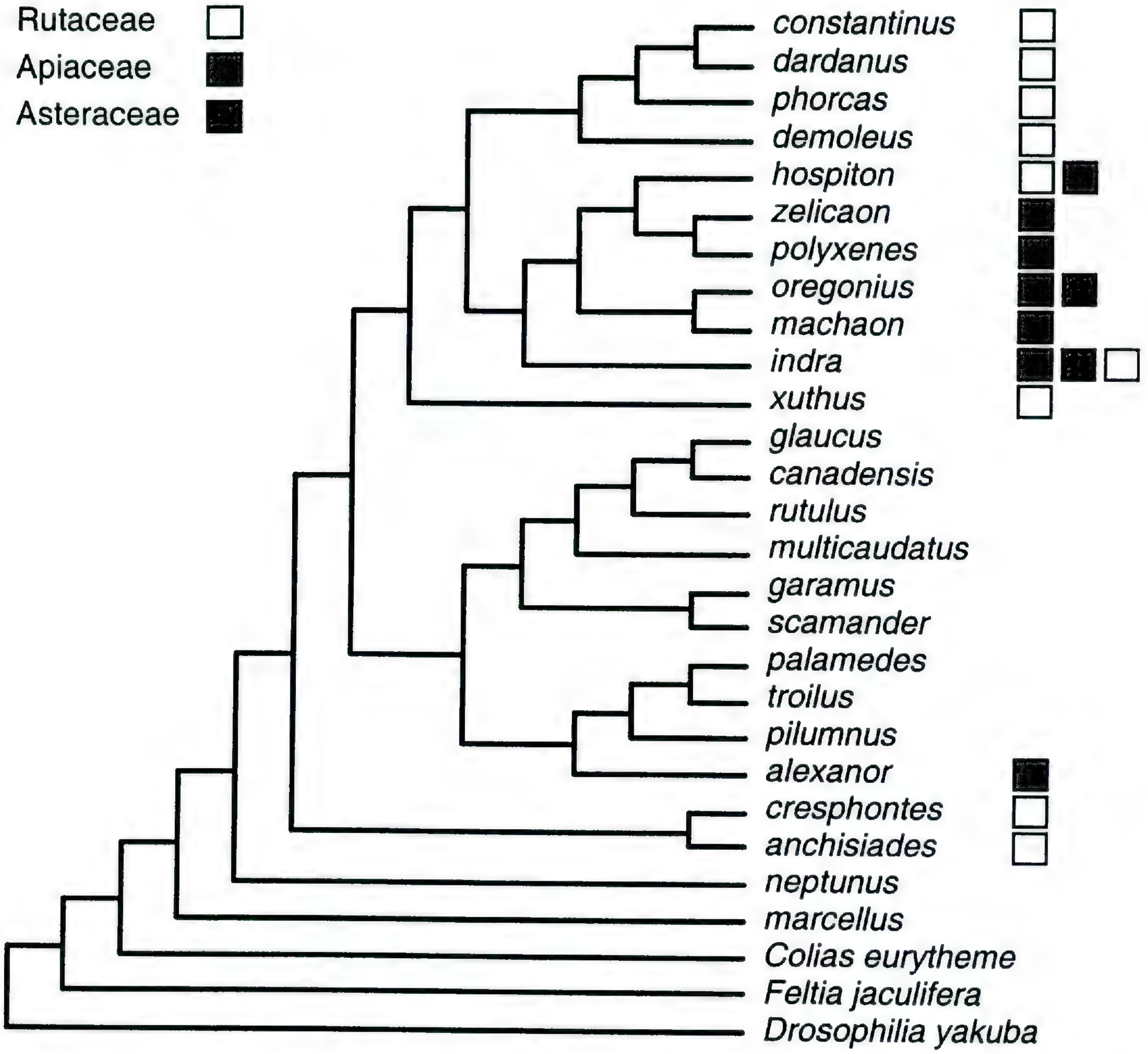


Figure 2. Phylogenetic relationships and hostplant associations in *Papilio* (Lepidoptera: Papilionidae) (modified from Caterino & Sperling, 1999).

within the genus *Depressaria,* half of the species (12 of 24) belong to the *douglasella* group, and no other group has more than five species (Hodges, 1974). Because *Agonopterix* and *Depressaria* are not sister groups, the Apiaceae must have been independently colonized at least twice by Depressariinae.

The results of this work on the North American fauna confirm that Rutaceae-feeding in the genus *Apachea* is ancestral to umbellifer-feeding in *Depressaria* and *Agonopterix.* The same pattern is suggested in European Depressariinae from phenetic studies (Hannemann, 1953, 1958), but no cladistic analysis of either North American or European Depressariinae existed at the time.

Papilio

Caterino and Sperling (1999) sequenced 2.3 kb of DNA from the mitochondrial cytochrome oxidase I and II genes for a number of species in the genus *Papilio.* They identified five major lineages—*machaon-phorcas-demoleus-xuthus, cresphontes-anchisiades, glaucus-garamas-scamander, troilus,* and *alexanor.* According to this analysis (Fig. 2), Apiaceae-feeding arose at least two times, in each case from Rutaceae-feeding ancestors (*alexanor, machaon* complex, *demoleus* group (*demodocus*)). The ancestral relationship of Rutaceae-feeders to Apiaceae/Asteraceae feeders in both the tribe Papilionini and the subfamily Depressariinae argues for a historical progression rather than simply opportunistic colonization of chemically related plants.

Papaipema

Papaipema species in the family Noctuidae are stem-borers, mostly of herbaceous plants. Most are oligophagous and a number feed exclusively on

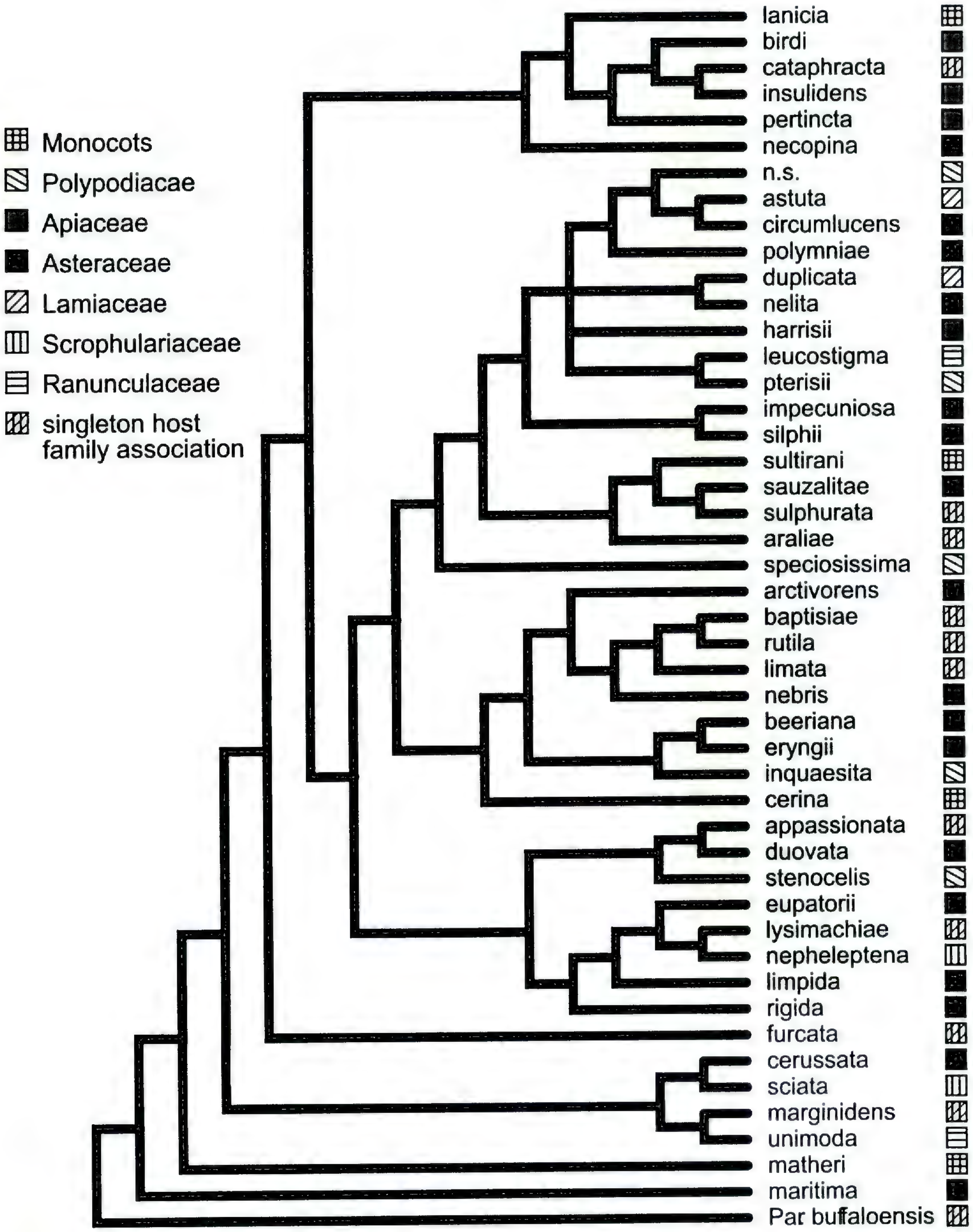


Figure 3. Phylogenetic relationships and hostplant associations in *Papaipema* (Lepidoptera: Noctuidae) (modified from Goldstein, 1999, and pers. comm.).

Apiaceae. Goldstein (1999) recently completed an examination of patterns of colonization of Apiaceae by species in the genus *Papaipema* (Fig. 3). Phylogenetic analysis suggests three independent origins of Apiaceae-feeding within *Papaipema*; no instances of Apiaceae-feeding can be found in apameine noctuids outside *Papaipema*. The Apiaceae feeders consist of *P. eryngii*, which appears as sister to an Asteraceae-feeding species; *P. harrisii*, which falls directly within an Asteraceae-feed-

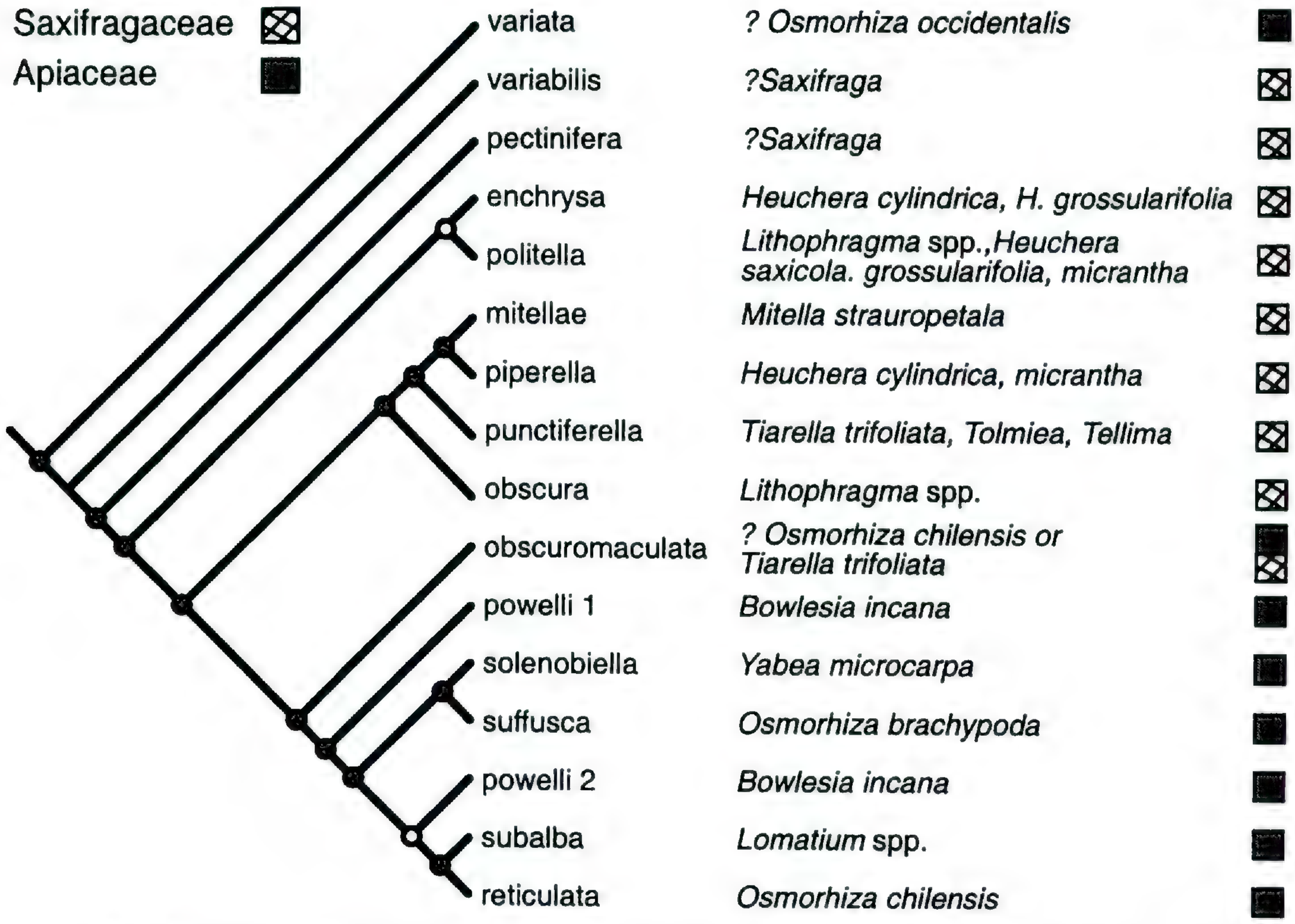


Figure 4. Phylogenetic relationships and hostplant associations in *Greya* (Lepidoptera: Prodoxidae Incurvarioidea) (modified from Brown et al., 1994).

ing clade; and the *P. birdi* group, which appears as paraphyletic on the tree, but which, depending on optimization, may also have arisen from an aster-feeding clade. It is noteworthy that the *birdi* group appears as paraphyletic to the generalist species *P. cataphracta*, which is recorded from many asteraceous species but only one apiaceous species, with that being a dubious record.

Greya

Brown et al. (1994) found that Apiaceae-feeding prodoxid moths form a phylogenetic unit. Apiaceae-feeders in this group are monophyletic; sister taxa are most likely associated with Saxifragaceae (Fig. 4). This host shift is very likely attributable to the fact that species in both host families occupy similar habitats in close geographical proximity; few if any chemical similarities are known to exist between these two families.

Euleia

Although the majority of Tephritidae (4000 species in 500 genera) are fruit or flower feeders, species in the genus *Euleia* are unusual in that they are leaf miners (as are all members of the subtribe Tripetina). Han and McPheron (1997) conducted an analysis of tephritid phylogeny using sequence data from the mitochondrial 16S ribosomal DNA (925 base pairs). Within the monophyletic Trypetini, Apiaceae-feeding is a derived trait and represents a shift from Asteraceae-feeding ancestors (Fig. 5).

Phytomyza

At least 15 species of *Phytomyza* (Diptera: Agromyzidae) are leaf miners of apiaceous plants. If there is a cladistic analysis of phylogeny, it is not readily available. However, it is interesting to note that at least four species (*P. albiceps*, *P. chrysanthemi*, *P. nigra*, and *P. obscurella*) are reported on both apiaceous and asteraceous hostplants.

Oreina

Dobler et al. (1996) examined hostplant switches in the chrysomelid genus *Oreina*, a Palearctic genus with approximately 24 species, most of which are alpine in distribution. Hostplant range includes *Oreina gloriosa* on a single species *Peucedanum ostruthium* (L.) W. Koch, to *O. bifrons* on two hosts in different tribes (*Peucedanum* and *Chaerophyl-*

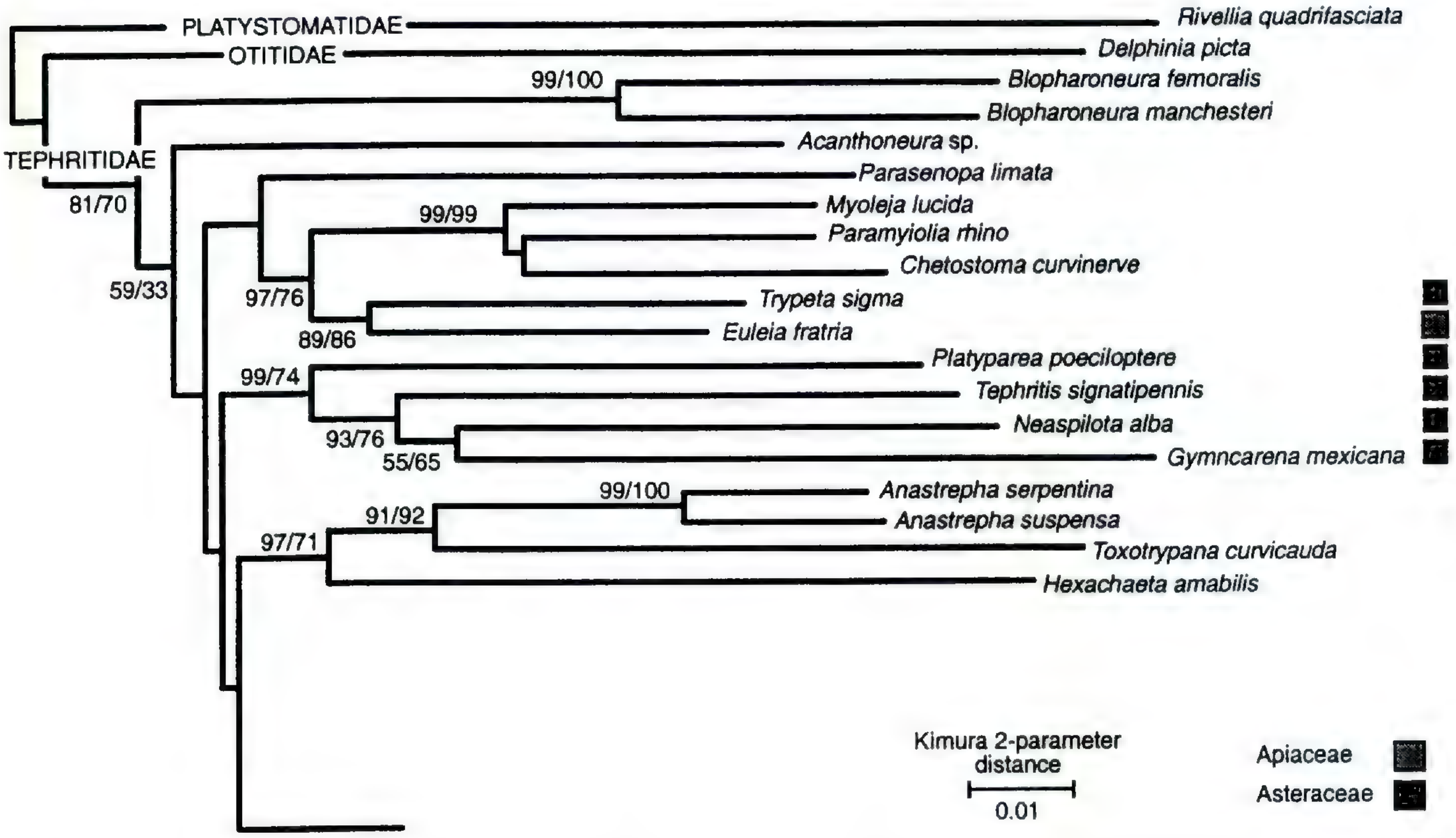


Figure 5. Phylogenetic relationships and hostplant associations in *Euleia* (Diptera: Tephritidae) (modified from Han & McPheron, 1997).

lum), to species on Apiaceae in three different tribes. The majority of species feed on Asteraceae in two tribes, Senecioneae and Cardueae. One species, *Oreina frigida,* accepts both Asteraceae and Apiaceae. These authors conducted a cladistic analysis of 18 allozyme loci (Fig. 6). Dobler et al. (1996) generated two most parsimonious trees, which demonstrated that the ancestral host for *Oreina* was most likely Asteraceae; Apiaceae occur in the diet of one oligophagous species and its occurrence in a derived clade represents an independent switch. The two asteraceous tribes utilized by these insects are phylogenetically "distant."

Comparisons Among Taxa

An examination of the phylogenetic history of colonization of Apiaceae across taxa reveals some inescapable patterns. Host shifts to the Apiaceae originate in only a narrow range of plant taxa. In four cases (twice within the depressariine elachistids and twice within the genus *Papilio*), Rutaceae-feeding is ancestral to Apiaceae-feeding, and in five cases (three in *Papaipema,* one in *Oreina,* and one in *Euleia*), Asteraceae-feeding is ancestral to Apiaceae-feeding. There are several possible explanations as to why this should be. Phylogenetic relationship can almost certainly be ruled out, however. The most likely sister family to the Apiaceae is the Araliaceae (Downie et al., 1998), and there is virtually no evidence of Araliaceae-feeding in any relative (distant or close) of an Apiaceae-feeder. By the same token, the sister family of Asteraceae, the hosts for immediate ancestors of a number of Apiaceae-feeders, is regarded by many (Bremer & Gustafsson, 1997) to be the Campanulaceae, again, a family absolutely absent from the host records of relatives of Apiaceae-feeders.

Another common explanation for host shifts is geographic or ecological proximity. Such an explanation is plausible for Asteraceae-Apiaceae shifts; both families are primarily temperate herbaceous and occupy similar habitats. There are many other families that share early successional habitats with the hostplants of these insect groups, including species of Lamiaceae, Brassicaceae, Polygonaceae, and Fabaceae, to name but a few, but host shifts from these groups to Apiaceae are non-existent. Geographic or ecological proximity is even less satisfying as an explanation for shifts to Apiaceae from Rutaceae, given that the Rutaceae consists primarily of trees and shrubs that are mostly tropical in origin.

Patterns of host shifts in Apiaceae associates parallel patterns of hostplant colonization among the butterflies (Janz & Nylin, 1998: plant classifications that follow according to this source). As do butterflies, a high proportion of depressariine oecophorid species use Rosidae as hostplants (all *Nites, Apachea,* and *Bibarrambla,* and 11 of 42 *Agonopterix*). If, in fact, the Amphisbatinae are an-

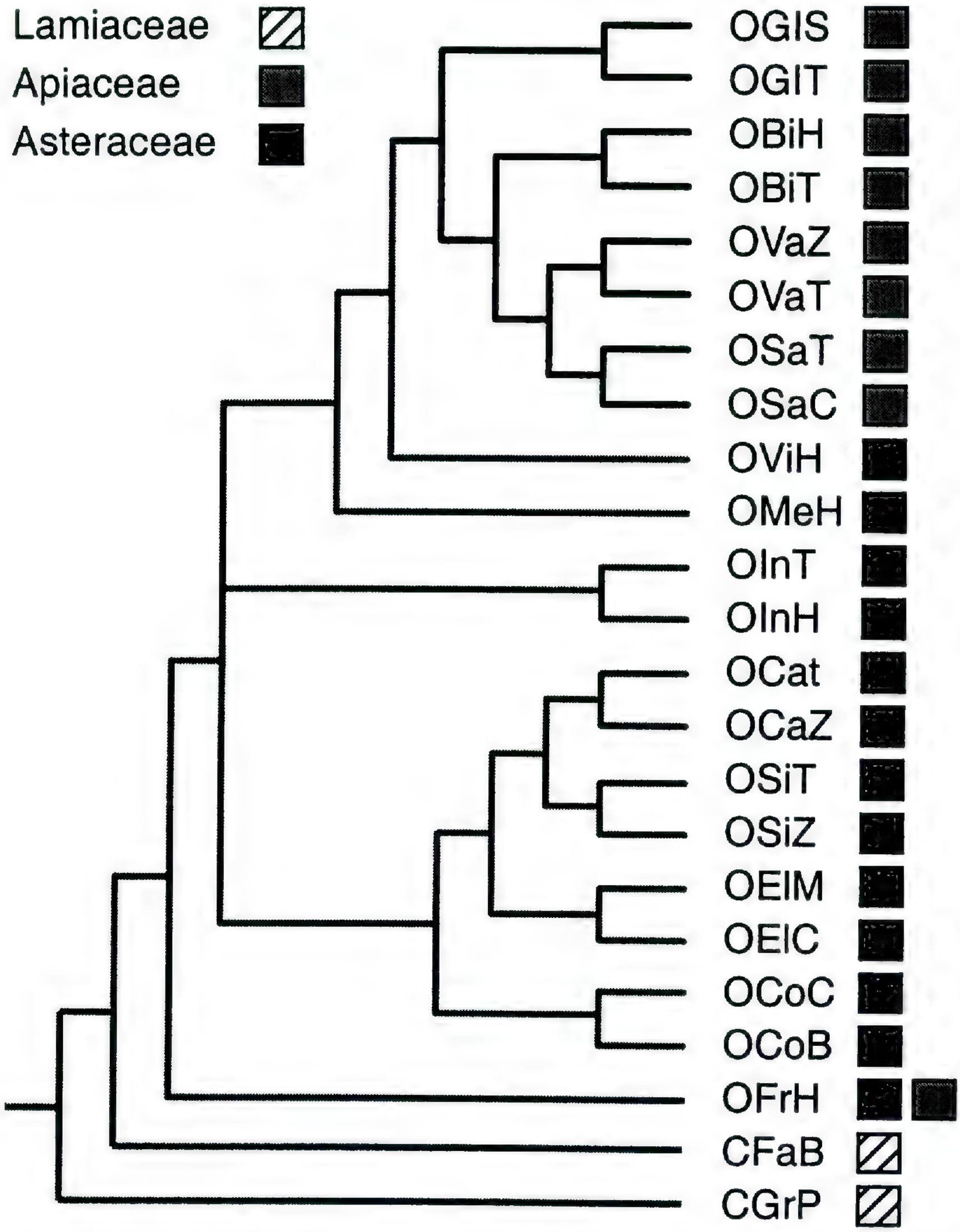


Figure 6. Phylogenetic relationships and hostplant associations in *Oreina* (Coleoptera: Chrysomelidae) (modified from Dobler et al., 1996). Species names include *O. bifrons* (O BiH, O BiT), *O. cacaliae* (O CaT, O CaZ), *O. coerulea* (O CoB, O CoC), *O. elongata* (O ElC), *O. frigida* (O FrH), *O. gloriosa* (O. GlS, O GlT), *O. intricata* (O InH, O InT), *O. melanocephala* (O MeH), *O. speciosa* (O SaC, O SaT), *O. speciossissima* (O SiT, O SiZ), *O. variabilis* (O VaT, O VaZ), *O. virgulata* (O ViH).

cestral to the Depressariinae-Ethmiinae clade (Passoa, 1995), then the Rosidae may be the ancestral hostplants; contemporary Amphisbatinae utilize representatives of Rosid 1A (Salicaceae), Rosid 1B (Ulmaceae), and Rosid 1C (Betulaceae, Fagaceae, Juglandaceae) clades. As is true for butterflies, genera associated with trees predominate; there is no obvious trend toward specialization on forbs at this level, and there is no evidence of a tendency for advanced insect taxa to feed on advanced plant taxa. Association with plants in the asterid 2 clade appears to have led to rapid speciation. The two most species-rich genera in the group, *Depressaria* and *Agonopterix*, are dominated by species that feed on Apiaceae and Asteraceae; all of the *Depressaria* species with known feeding habits are found on these plant families, and 19 of 42 *Agonopterix* species feed predominantly or exclusively on these plants.

Chemistry provides an extremely attractive organizing principle to account for patterns of colonization and diversification in virtually all taxa associated with Apiaceae. The distinctive groups of secondary chemicals shared by Asteraceae and Apiaceae include cyclitols, isoeugenol epoxides, germacranolides, guaianolides, and eudesmanolide sesquiterpene lactones, 2-methyl chromones, petroselinic acid, triterpene sapogenins, polyacetylenes

Table 3. Phytochemical characteristics of Apiaceae, plants utilized by Apiaceae-feeders, and sister taxa to plant groups utilized by Apiaceae-feeders (phytochemical data from Hegnauer, 1971).

Phytochemical	Api-aceae	Arali-aceae	Ruta-ceae	As-tera-ceae	Cam-panu-laceae
Essential oils	x	x	x	x	
Furanocoumarins	x		x		
Pyranocoumarins	x		x		
Isopentenyl coumarins	x		x	x	
Polyacetylenes	x	x		x	x
Triterpenic saponins	x	x		x	x
2-methyl chromones	x		x	x	
Guaianolides	x			x	
Germacranolides	x			x	
Petroselinic acid	x				
Tannins lacking	x	x		x	x
Lignans	x	x	x	x	

(although in Asteraceae these tend to be cyclic, with aromatic or heteroaromatic terminal groups), and coumarins. These families also have the absence of certain chemicals in common; both lack ellagitannins, proanthocyanidins, and iridoids. The chemical resemblances between these families are all the more striking considering the generally lower level of chemical resemblance between sister taxa. The Asteraceae have more chemistry in common with the Apiaceae than with the Campanulaceae, widely regarded as the sister group; by the same token, although Apiaceae and its sister group Araliaceae share many chemicals, there are more different types of secondary compounds in common between Apiaceae and Asteraceae (Table 3).

Thus, the question remains—why do distantly related plant taxa have more similar chemistries than closely related taxa? Convergence in morphological traits in plants arises as a result of common ecologies—desert-dwelling plants have reduced leaf area to conserve water, and bee-pollinated plants have zygomorphic aromatic blue flowers to attract their mutualists. Similar ecologies, including similar herbivore faunas, could account for similarities in overall chemical profile. These patterns are actually suggestive of reciprocity—it is difficult otherwise to provide a scenario to account for such remarkable convergences (other than common ancestry, which, according to systematists, is not the likely explanation). Chemical innovation can lead to adaptive radiation—and it can also lead to colonization by preadapted species. In a study aimed at determining the basis for host shifts by species of flea beetle in the genus *Blepharida,* Becerra and Venable (1999) found that geographic similarity and phylogenetic relatedness could not account as well for the patterns observed as could hostplant chemistry; for specialists within the genus, plant chemical similarity appears to be the best explanation for shifts within this group.

Similar selection pressures can increase the probability of parallel evolution. The key, then, is the preadaptation of the insects to co-occurring groups of chemicals. Considerable evidence exists that co-occurring chemicals can interact in additive and synergistic ways (Berenbaum, 1985; Berenbaum & Zangerl, 1996); these combinations of chemicals thus can exert selection pressures quite unlike those exerted by component chemicals in isolation. There is also experimental evidence that insects adapt to combinations of chemicals in distinctive ways. Methylenedioxyphenyl compounds in Apiaceae (e.g., myristicin) and Asteraceae (e.g., sesamin), for example, are associated with potentiation of furanocoumarins; these MDP compounds act as inhibitors of cytochrome P450 monooxygenases, the enzymes largely responsible for furanocoumarin detoxification in herbivorous insects. Specialists on Apiaceae show greater resistance to the P450-inhibiting effects of these compounds, and concomitantly greater tolerance of co-occurring furanocoumarins, than do insects that normally do not encounter these compounds together (Neal & Wu, 1994; Zumwalt & Neal, 1993). Thus, combinations of toxins and synergists present a different toxicological challenge to insects, and counteradaptation presents a different resistance challenge to plants, in which a narrower range of chemistries may be available to overcome resistance. Similar faunas can thus be expected to select for plants with similar kinds of chemistry.

Colonization of a new hostplant by an insect is possible only if parents can locate the hosts and immature feeding stages are equipped metabolically to deal with the hosts. It is thus not altogether surprising that insects sharing certain chemically distinct hostplants colonize new hostplants in similar fashion, and in doing so they jointly act to affect evolution in those hostplants. In nature, then, as in human culture, it appears inexorably that there really are a limited number of ways to eat an umbellifer—a limited number of evolutionary approaches—due to the unique nature of its chemistry.

Literature Cited

Arber, A. 1938. Herbals: Their Origin and Evolution A Chapter in the History of Botany (1470–1670). Cambridge Univ. Press, Cambridge.

Becerra, J. X. & D. L. Venable. 1999. Macroevolution of insect-plant associations: The relevance of host biogeography to host affiliation. Proc. Natl. Acad. Sci. U.S.A. 96: 12626–12631.

Berenbaum, M. 1983. Coumarins and caterpillars: A case for coevolution. Evolution 37: 163–179.

———. 1985. Brementown revisited: Allelochemical interactions in plants. Recent Advances Phytochem. 19: 139–169.

———. 1990. Evolution of specialization in insect-umbellifer associations. Annual Rev. Entomol. 35: 319–343.

———. 1991. Coumarins. Pp. 221–249 *in* G. Rosenthal & M. Berenbaum (editors), Herbivores: Their Interactions with Secondary Plant Metabolites. 2nd Ed. Vol. 1. Academic Press, New York.

——— & S. Passoa. 1999. Generic phylogeny of North American Depressariinae (Lepidoptera: Elachistidae) and hypotheses about coevolution. Ann. Entomol. Soc. Amer. 92: 971–986.

——— & A. R. Zangerl. 1996. Phytochemical diversity: Adaptation or random variation? *In:* J. Romeo et al. (editors), Phytochemical Diversity and Redundancy in Ecological Interactions. Recent Advances Phytochem. 30: 1–24. Plenum Press, New York.

Bohlmann, F. 1971. Acetylenic compounds in the Umbelliferae. Pp. 279–291 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, New York.

Bremer, K. & M. H. G. Gustafsson. 1997. East Gondwana ancestry of the sunflower alliance of families. Proc. Natl. Acad. Sci. U.S.A. 94: 9188–9190.

Brown, J. M., O. Pellmyr, J. N. Thompson & R. G. Harrison. 1994. Mitochondrial DNA phylogeny of the Prodoxidae (Lepidoptera: Incurvarioidea) indicates rapid ecological diversification of yucca moths. Ann. Entomol. Soc. Amer. 87: 795–802.

Brues, C. T. 1920. The selection of food-plants by insects, with special references to lepidopterous larvae. Amer. Naturalist 54: 313–332.

Burki, C. & W. Nentwig. 1997. Comparison of herbivore insect communities of *Heracleum sphondylium* and *H. mantegazzianum* in Switzerland (Spermatophyta: Apiaceae). Entomol. Generalis 22: 147–155.

Caterino, M. S. & F. A. H. Sperling. 1999. *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Molec. Phylogenet. Evol. 11: 122–137.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y.-L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedrén, B. S. Gaut, R. K. Jansen, K.-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q.-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan & V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.

Constance, L. 1971. History of the classification of Umbelliferae (Apiaceae). Pp. 1–8 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, New York.

Culpepper, N. 1616–1654 (Reprint 1955). The Complete Herbal. London.

Dethier, V. 1941. Chemical factors determining the choice of food plants by *Papilio* larvae. Amer. Naturalist 75: 61–73.

Dobler, S., P. Mardulyn, J. M. Pasteels & M. Rowellrahier. 1996. Host-plant switches and the evolution of chemical defense and life history in the leaf beetle genus *Oreina*. Evolution 50: 2373–2386.

Downie, S. R., S. Ramanath, D. S. Katz-Downie & E. Llanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid RPOC1 intron sequences. Amer. J. Bot. 85: 563–591.

Ehrlich, P. & P. H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution 18: 586–608.

Fairbairn, J. W. 1971. The alkaloids of hemlock (*Conium maculatum* L.) (or *Conium maculatum* L.: The odd man out). Pp. 361–368 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, New York.

Farrell, B. D. & C. Mitter. 1998. The timing of insect/plant diversification: Might *Tetraopes* (Coleoptera: Cerambycidae) and *Asclepias* (Asclepiadaceae) have coevolved? Biol. J. Linn. Soc. 63: 553–577.

———, ——— & D. J. Futuyma. 1992. Diversification at the insect-plant interface. Bioscience 42: 34–42.

Fraenkel, G. 1959. The raison d'être of secondary plant substances. Science 129: 1466–1470.

French, D. H. 1971. Ethnobotany of the Umbelliferae. Pp. 385–393 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, New York.

Funk, D., D. J. Futuyma, G. Orti & A. Meyer. 1995. A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): New evidence from mitochondrial DNA. Evolution 49: 1008–1017.

Futuyma, D. J. 1983. Selection factors in the evolution of host choice by phytophagous insects. Pp. 227–244 *in* S. Ahmad (editor), Herbivorous Insects: Host-seeking Behavior and Mechanisms. Academic Press, New York.

Goldstein, P. Z. 1999. Molecular Systematics and the Macroevolution of Host Plant Use in the Endophagous Moth Genus *Papaipema* Smith. Doctoral Dissertation, University of Connecticut, Storrs.

Han, H.-Y. & B. A. McPheron. 1997. Molecular phylogenetic study of Tephritidae (Insecta: Diptera) using partial sequences of the mitochondrial 16S ribosomal DNA. Molec. Phylogenet. Evol. 7: 17–32.

Hannemann, J. H. 1953. Naturliche Gruppierung der europaischen Arten der Gattung *Depressaria* s.l. (Lep. Oecoph.). Mitt. Zool. Mus. Berlin 29: 270–372.

———. 1958. Die Gruppierung weiterer Depressarian nach dem Bau ihrer Kopulationsorgane: Teil 1. Mitt. Zool. Mus. Berlin 34: 3–47.

Harborne, J. B. 1971. Flavonoid and phenylpropanoid patterns in the Umbelliferae. Pp. 293–314 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, New York.

Hedge, I. C. 1973. Umbelliferae in 1672 and 1972. Notes Roy. Bot. Gard. Edinburgh 32: 151–158.

Hegnauer, R. 1971. Chemical patterns and relationships of Umbelliferae. Pp. 279–292 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, New York.

Hodges, R. W. 1974. Gelechioidea Oecophoridae. Fascicle 6.2 *in* R. B. Dominick, D. C. Ferguson, J. G. Fran-

clemont, R. W. Hodges & E. G. Munroe, The Moths of America North of Mexico. E. W. Classey, London.

———, T. Dominick, D. R. Davis, D. L. Ferguson, J. C. Franclemont, E. G. Munroe & J. A. Powell. 1983. Check List of the Lepidoptera of America North of Mexico. Wedge Entomological Research Foundation, Cambridge.

Janz, N. & S. Nylin. 1998. Butterflies and plants—A phylogenetic study. Evolution 54: 486–502.

Jermy, T. 1993. Evolution of insect-plant relationships—A devil's advocate approach. Entomol. Exp. Appl. 66: 3–12.

Marquis, R. J. 1992. Selective impact of herbivores. Pp. 301–325 *in* R. S. Fritz & E. L. Simms (editors), Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics. Univ. Chicago Press, Chicago.

Menken, S. B. J. 1996. Patterns and process in the evolution of insect-plant associations—*Yponomeuta* as an example. Entomol. Exp. Appl. 80: 297–305.

Mitter, C. & D. R. Brooks. 1984. Phylogenetic aspects of coevolution. Chapter 4, pp. 65–98 *in* D. J. Futuyma & M. Slatkin (editors), Coevolution. Sinauer, Sunderland, Massachusetts.

Nakamura, M. 1981. Key to the classification of the Japanese lepidopterous pupae. Lepidopterological Soc. Japan 32: 1–12.

Neal, J. J. & D. Wu. 1994. Inhibition of insect cytochromes P450 by furanocoumarins. Pest. Biochem. Physiol. 50: 43–50.

Passoa, S. 1995. Larval and pupal systematics of Nearctic Amphisbatinae and Depressariinae (Lepidoptera: Oecophoridae). Doctoral Dissertation, University of Illinois, Urbana.

Rohde, E. S. 1922. The Old English Herbals. London.

Schoonhoven, L. M., T. Jermy & J. J. A. van Loon. 1998. Insect Plant Biology: From Physiology to Evolution. Chapman and Hall, London.

Stahl, E. 1888. Pflanzen und Schnecken. Jenaische Z. Med. 22: 557–684.

Thompson, J. N. 1999. What we know and do not know about coevolution: Insect herbivores and plants as a test case. Pp. 7–30 *in* H. Olff, V. K. Brown & R. H. Drent (editors), Herbivores: Between Plants and Predators. Blackwell, Oxford.

Zumwalt, J. G. & J. J. Neal. 1993. Cytochromes P450 from *Papilio polyxenes*: Adaptations to host plant allelochemicals. Comp. Biochem. Physiol. 106C: 111–118.

THE GENUS *STRIGA* (SCROPHULARIACEAE) IN AFRICA[1]

Kamal I. Mohamed,[2] Lytton John Musselman,[3] and Charles R. Riches[4]

ABSTRACT

Twenty-eight species and six subspecies of the hemiparasitic genus *Striga* Lour., witchweed, occur in Africa. Twenty-two species are endemic. Witchweeds occur throughout the continent with greatest diversity in the grasslands and savannas north of the equator. *Striga gesnerioides* (Willd.) Vatke and *S. hermonthica* (Del.) Benth. have developed host-specific strains that may have distinct morphotypes. We accord these no formal taxonomic status. *Striga hermonthica* and *S. asiatica* (L.) Kuntze, limited to agroecosystems, may be recently derived from non-ruderal species. *Striga hirsuta* Benth. and *S. lutea* Lour., sometimes treated as conspecific with *S. asiatica*, are recognized as distinct species restricted to natural ecosystems. *Striga linearifolia* Hepper, with its strongly bilabiate corolla and perennial habit, is considered as a subspecies of *S. bilabiata* (Thunb.) Kuntze. *Striga baumannii* Engl. and *S. fulgens* Hepper are excluded from the genus. *Striga baumannii* has tuberous roots and a ligneous calyx and may represent a distinct genus. *Striga fulgens*, with free petals, is aligned with *Chascanum* E. Mey. in the Verbenaceae.

Key words: Africa, Scrophulariaceae, *Striga*, witchweed.

Globally, witchweeds have a greater impact on human welfare than any other parasitic angiosperms because their hosts are subsistence crops in areas marginal for agriculture. They are also among the most specialized of all root-parasitic Scrophulariaceae (or Orobanchaceae, depending on how the families are circumscribed). Some members of the Scrophulariaceae are holoparasitic (without chlorophyll); most are hemiparasites (with chlorophyll). *Striga* combines life styles of both with a holoparasitic seedling and a green, chlorophyll-containing emergent plant. These furtive pathogens attack their hosts underground and, by the time the flowering stem of the parasite emerges and is evident, the crop is damaged. This occult behavior may be the source of the Latin name, "Striga," meaning "hag" or "witch." In this way, hosts are "bewitched" because the farmer is unaware of the parasite until it comes up. Due to the serious injury they inflict, witchweeds have been the targets of many control strategies. Helpful reviews of control measures and other aspects of witchweed biology are found in Sand et al. (1990), Sauerborn (1991), Parker and Riches (1993), and Press and Graves (1995).

Because witchweeds are parasitic and not totally dependent upon leaves, no exclusively vegetative stems are produced. In other words, each stem bears an inflorescence. Flowers are usually showy, in some cases spectacular. Most species that have been studied are autogamous (e.g., Musselman & Hepper, 1986), although *S. hermonthica* and *S. aspera* (Willd.) Benth. are outcrossers (Aigbokhan et al., 1998). Seed production is prodigious. Conditioning, germination, parasitic contact, and penetration are mediated by elegant systems of chemical communication between host and parasite (Parker & Riches, 1993; Press & Graves, 1995; Maass, 1999).

Witchweeds tolerate a relatively wide range of climatic and soil conditions. They grow in areas with annual rainfall ranging from 25 to 150 cm per year with a decrease in the severity of infestation in areas with higher rainfall (Musselman & Ayensu,

[1] This work is based mainly on the dissertation research of K. I. Mohamed that was supported, in part, by grant USDA 58-319R-5-0006. Research conducted in Nigeria was supported by grant AID DHR 5600-G-00-1021-00. Many people have assisted in our studies of the genus over the past several years. We thank E. Aigbokhan, A. E. Awad, F. F. Bebawi, D. Berner, R. Eplee, H. Glen, F. N. Hepper, C. Jarvis, D. Kenfack, M. O. Khidir, R. Moberg, D. L. Nickrent, R. Norris, S. N. C. Okonkwo, C. Parker, A. Pieterse, K. V. Ramaiah, J. Ransom, A. Raynal-Roques, S. B. Safa (deceased), M. J. Vasudeva Rao, P. J. Terry, J. Thieret, M. Thulin, J. H. Visser (deceased), E. P. Weber, and A. Wolfe. Appreciation is extended to the curators and staff of BRE, BM, GH, K, MO, ODU, S, US, and UPS for the loan of specimens.

[2] Department of Biology, State University of New York College at Oswego, Oswego, New York 13126-3501, U.S.A. mohamed@oswego.edu.

[3] Mary Payne Hogan Professor of Botany, Old Dominion University, Norfolk, Virginia 23529-0266, U.S.A. lmusselm@odu.edu.

[4] Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Chatham, Kent ME4 4TB, U.K. charlie.riches@bbsrc.ac.uk.

1984). Optimum temperature for seed germination is between 30°C and 40°C, with no germination at 15°C or lower, or higher than 45°C (see review of literature in Parker & Riches, 1993).

Phenology among the different species of *Striga* varies. In a given area, there is a progression of appearances from the onset of the rains to the end of the rainy season. For example, in the Southern Kordofan region of Sudan, the first *Striga* to appear is *S. forbesii* Benth., followed by *S. passargei* Engl.→ *S. aspera*→ *S. gesnerioides*→ *S. hirsuta*→ and lastly, *S. hermonthica.*

In general, low soil fertility, nitrogen deficiency, well-drained soils, and water stress accentuate the severity of *Striga* damage to the host. These are typically the environmental conditions for *Striga*-hosts in the semiarid tropics (Sauerborn, 1991; Parker & Riches, 1993). *Striga* usually causes less damage to hosts growing on fertile, deep soils.

Striga is distinguished from other genera in the subfamily Rhinanthoideae of the Scrophulariaceae by bilabiate corollas with a pronounced bend in the corolla tube, and unilocular anthers. The corolla feature distinguishes *Striga* from related *Buchnera* L. in which nine *Striga* species were once included. Based on this feature, the genus *Striga* was erected by the Italian botanist Loureiro in 1790 as distinct from *Buchnera,* which has a salverform corolla. Little data are available on seed structure in *Buchnera,* but Musselman and Mann (1976) reported secondary ornamentation of *Buchnera americana* L. similar to that of *Striga asiatica. Buchnera* germination and seedling development are also much less specialized (Parker & Riches, 1993). Lastly, *Buchnera* is found in both the New and Old Worlds, while *Striga* is strictly Old World.

Recent molecular work supports the long-assumed relationship between *Striga* and *Buchnera.* Olmstead et al. (in press), using three plastid genes, show *Buchnera floridana* Gandoger (= *B. americana*) and *S. asiatica* as sister taxa. Further work is needed to determine the relationship between *Alectra* and *Striga.* Unlike *Buchnera, Alectra* species require a germination stimulant and have a holoparasitic seedling stage.

All witchweeds are African except the following: *S. curviflora* Benth. (Australia, New Guinea), *S. densiflora* (Benth.) Benth. (India), *S. masuria* (Buch.-Ham. ex Benth.) Benth. (= *S. flava* Miq.?, *S. esquirolia* Lév.?) (China, India, Indonesia), *S. multiflora* Benth. (Australia), *S. parviflora* Benth. (Australia), and *S. sulphurea* Dalz. ex Dalz. & Gibbs (India). Our understanding of the genus in Africa requires a better knowledge of these species.

Taxonomic studies at the genus and tribal level are few. Wettstein (1891) divided *Striga* into two sections: the *Pentapleurae,* which have a 5-ribbed calyx, and the *Polypleurae,* characterized by approximately 10-calyx ribs. Later, Engler (1897) created the section *Tetrosepalum* to include *S. baumannii.* Saldanha (1963) noted that the number of ribs is inconsistent within species. In spite of this, rib number has remained as the only infrageneric character.

Figures 1 and 2 illustrate the general features of witchweeds.

Following is a summary of features helpful in determining *Striga* species.

1. Duration. *Striga* species are annuals or perennials. Duration may help identify plants in the field, but it is of limited value for herbarium specimens, especially when roots are lacking. All agronomically important species are annuals, perhaps because their hosts are also annuals. The exception is sugarcane (*Saccharum officinarum* L.), which is perennial. In natural grasslands witchweed species do not seem to affect their hosts the same as cultivated crops; damage is not evident. Perennial species produce fleshy underground rhizomes or tubers that bear scale leaves from which new stems arise annually. Generally, perennial species have reduced or narrow stem leaves. See Raynal-Roques (1993) for a helpful discussion of duration in witchweeds.

2. Stem shape. Three stem types can be distinguished. Terete stems are round in cross section, obtusely square stems are square with blunt corners, and winged stems are square and acutely angled. A furrowed stem has grooves parallel to the long axis.

3. Indumentum. Indumentum types, both surface features and trichomes, are diverse. Many species have scabrous leaf and stem surfaces at maturity. The most common trichome types include: glandular trichomes; stiff hairs (hispid); long soft hairs (pubescent or pilose); long stiff hairs (ciliate) (Neumann et al., 1996).

4. Leaf lobing, dentation. Leaves of most species are unlobed, and only a few have teeth. Venation may extend to the tips of leaves in a few species.

5. Inflorescence. Inflorescence types include solitary flowers, spikes, and racemes. The length of the inflorescence compared to the vegetative part of the stem (longer or shorter); compactness of the flowers (lax or dense); and bract size and shape (similar to or different from stem leaves) are also helpful distinguishing features. Bracteoles are present just above the floral bract. Flowers can be opposite or alternate on the inflorescence. In almost

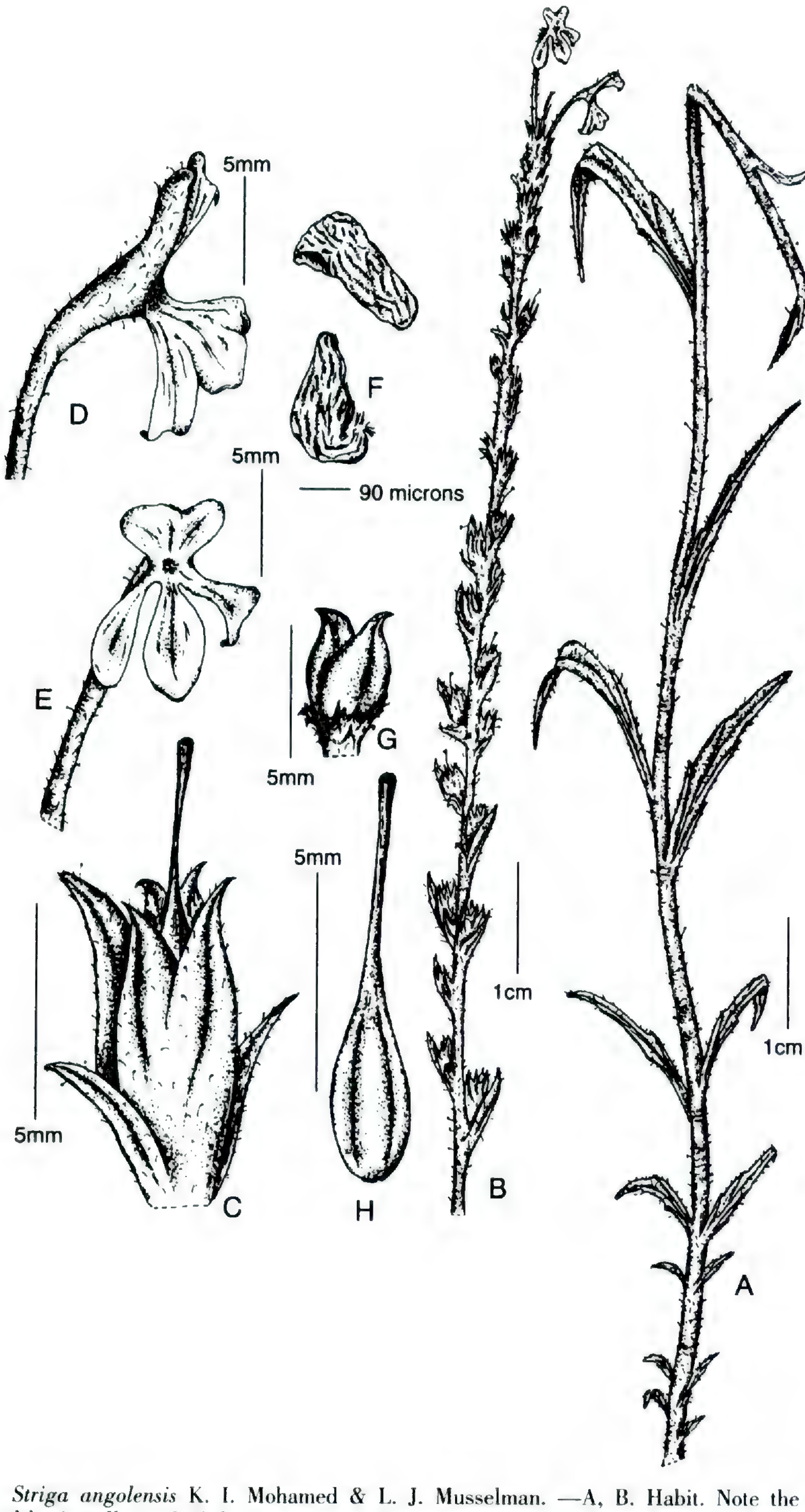


Figure 1. *Striga angolensis* K. I. Mohamed & L. J. Musselman. —A, B. Habit. Note the reduction of leaves acropetally and basipetally. —C. Calyx. Two bracteoles are evident, with the much larger bract on the left. This calyx has four lobes. —D, E. Corolla. —F. Seeds. —G. Capsule. —H. Pistil. (*R. G. N. Young 1365*, MO). Drawing by Karillos Karillos in Mohamed and Musselman, Brittonia 49: 118–121. 1997.

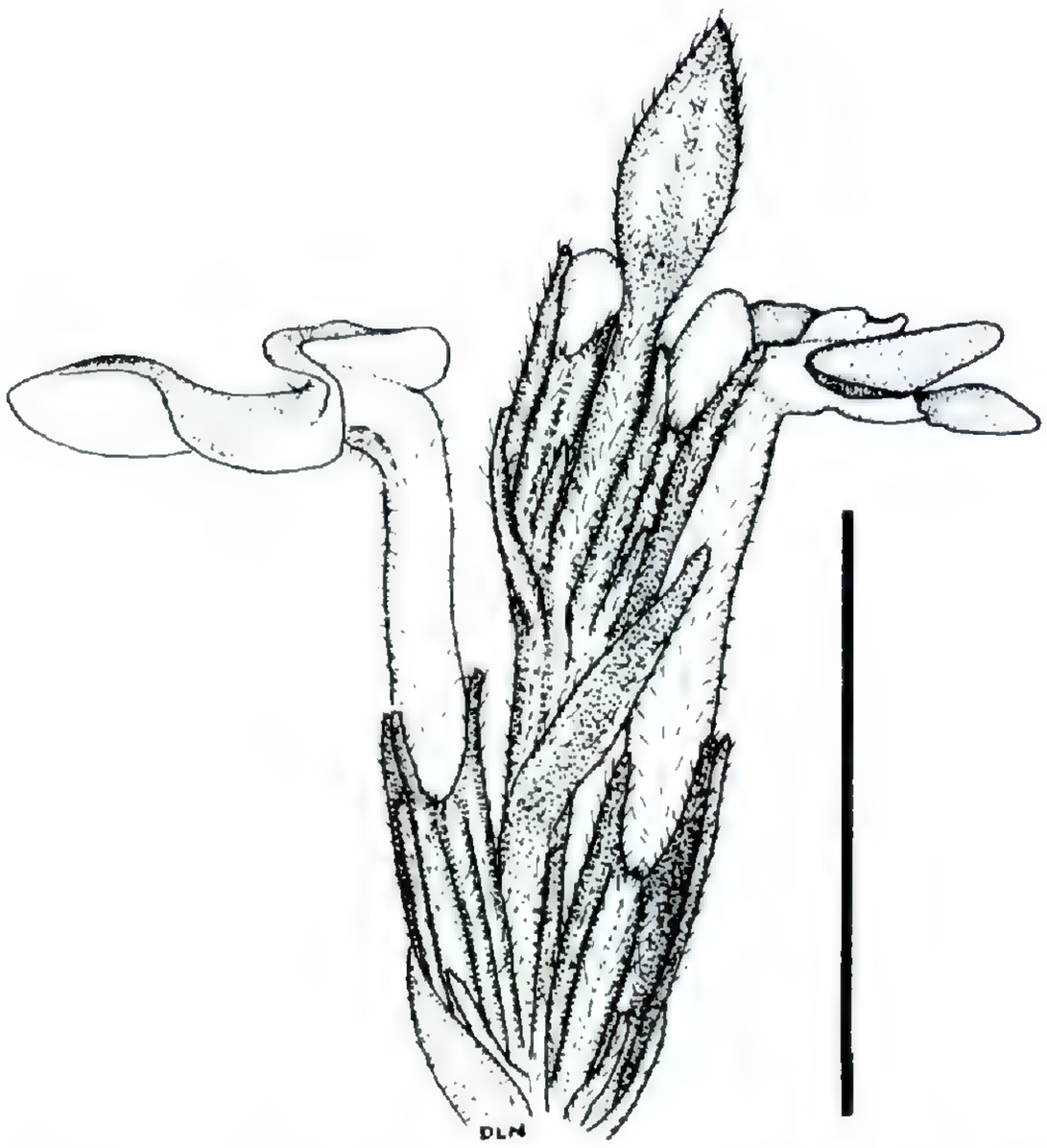


Figure 2. *Striga hirsuta* (Benth.) Benth. Note bract and bracteole, lower left. *Musselman & Mansfield* 5531 (ODU). Drawing by Daniel L. Nickrent from Musselman et al., Sida 8: 196–201. 1979. (Originally determined as *S. asiatica* (L.) Kuntze.) Scale = 1 cm.

all species the 2 to 4 pairs of lower bracts at the base of the inflorescence are longer than the upper bracts and are described separately.

6. Calyx. Calyx lobes can be equal or subequal. In some species the adaxial lobe is reduced.

7. Corolla color and tube bend. Most witchweed corollas are red or white, but they can also be salmon, orange, or yellow. The bend in the corolla tube can be positioned distally or proximally. If proximal, it may be within the calyx teeth. If distal, it is closer to the corolla limb. Fragrance has been reported only in *S. hermonthica* (Musselman & Hepper, 1986).

8. Hosts. Witchweeds have one of the narrower host ranges of any parasitic Scrophulariaceae. Not surprisingly, hosts of agronomically important species are better documented than hosts of native species. Most *Striga* species, however, have not been critically examined for host range and host specificity. Except for *S. gesnerioides*, which is restricted to dicots, these hosts are all Poaceae with the odd connection to non-grasses noted, disproportionately, by collectors. Great caution is advised when ascribing any form of host specificity to root parasites. It is essential that the host-parasite connection be located, not that hosts be inferred from proximity. Further, it is virtually impossible to determine how many different plants, perhaps different species, are attacked by a single witchweed.

Too few studies on other features of witchweeds are available, including cytology. Reported chromosome numbers have varied widely, from $n = 18$ to $n = 40$ (reviewed in Aigbokhan et al., 1998). Careful cytological studies are needed to clarify the counts. Ultrastructural studies are also limited. Pollen has few useful features, at least in the species studied (DeLeonardis, 1986; Minkin & Eshbaugh, 1989). Current taxonomy is based almost exclusively on morphology.

Seed size and structure have been used to separate *S. angustifolia* from other species because of its larger seed size and its germination without a host stimulus (e.g., Parker & Riches, 1993). Secondary ornamentation of *S. angustifolia* is also distinct from other species (Musselman & Parker, 1981). However, in a survey of nine species, no other consistent seed ornamentation features were found to separate species (Musselman & Parker, 1981).

A conspectus of the genus on a continent-wide basis is important for agricultural researchers, among others, to enable the determination of new parasite problems. Certainly, future expansion of food crop species possibly susceptible to parasitism by witchweed should take into account indigenous *Striga* species that could potentially attack crops. There are several examples where this has occurred. These include maize planted in the KweKwe region of Zimbabwe (Knepper et al., 1991) where *S. forbesii* occurred. Another is the planting of sugarcane in Ethiopia, which was attacked by *S. latericea* Vatke (Parker, 1988). Moreover, meaningful quarantine measures depend on knowledge of the distribution of the parasite.

A study of *Striga* taxonomy invites comparison with the taxonomy of the other, agronomically significant genus in the family (assuming parasitic Scrophulariaceae and Orobanchaceae are monophyletic, e.g., Nickrent et al., 1998; Young et al., 1999). This is *Orobanche*, the genus of broomrapes.

Historically, broomrapes have suffered from extreme splitting and the establishment of varieties, forms, and even subforms based largely on herbarium studies with little consideration of host-induced variation. Thus, a plethora of names encumbers the taxonomy of the group. Unlike *Orobanche*, the cytology of witchweeds needs much additional work. Based on the limited data available, aneuploidy does not play a major role in witchweed speciation and variability. Some groups of broomrapes, on the other hand, exhibit aneusomaty, polyploidy, and parthenogenesis (Cubero, 1996). In short, the taxonomy of witchweeds is less daunting than that of broomrapes.

Despite the well-known impact of *Striga* on sub-

sistence crops in Africa, there is no single reference to the taxonomy and distribution of African taxa. The purpose of this paper is to describe all the reported taxa in Africa, map their distribution, record notes on their biology, and provide a key for their identification.

Maps of *Striga* collection sites were constructed using the *Britannica Atlas*. Collection sites were plotted on a large computer-generated map (Anonymous, 1996).

Striga Lour., Fl. Cochinchinensis 1: 22. 1790.
TYPE: *Striga lutea* Lour.

Annual or perennial, herbaceous hemiparasites. Stem: stiffly erect, square or terete, ridged in some species. Indumentum: hirsute or pilose, scabrid, glandular pubescent; rarely glabrate. Leaves: opposite or subopposite, rarely alternate, reduced to small scales at the base of the stem in all species. Inflorescence: dense or lax spike or raceme; rarely solitary. Calyx: tubular, with 4 to 8, usually 5, equal or subequal lobes. Corolla: divided into a tube and a limb, the tube cylindrical with an acute bend, the limb 2-lipped, with lobes fused at least at their bases to form a bilabiate corolla with 3 lower and 2 upper lips. Stamens: 4, didynamous, anthers dorsifixed, unilocular, dehiscence loculicidal. Pollen: with 3–4 colpi, 15–30 μm with 2 nuclei at shedding. Style: terete, elongate, and persistent. Capsule: oblong or subovoid, placentation axillary, dehiscence loculicidal. Seeds [except in *S. angustifolia* (Don) Saldanha]: dust-like, 0.35 × 0.20 mm, with prominent ridges that are ornamented. Germination: hypogeal and cryptocotylar [except in *S. angustifolia*]. Chromosome numbers ranging from $n = 18$ to $n = 40$. Hosts: usually Poaceae; few species restricted to dicots.

KEY TO THE AFRICAN *STRIGA* SPECIES

1. Calyx 4- to 5-ribbed (sect. *Pentapleurae*) 2
1′. Calyx 10- or more ribbed (sect. *Polypleurae*) 14
2(1). Lobes of lower lip fused to >50% of their length forming a strongly bilabiate corolla 3
2′. Lobes of lower lip fused to <50% of their length forming a weakly bilabiate corolla 4
3(2). Plant glabrate with few hairs on leaf margins and bracts; calyx ≤4 mm long ..... 1. *S. aequinoctialis*
3′. Plant pubescent or hispid; calyx > 4 mm long ..... 6. *S. bilabiata*
4(2). Leaf prominently 3-veined, leaf margin toothed 5
4′. Leaf veins obscure, not apparent, leaf margin entire 6
5(4). Calyx lobes twice as long as tube; leaf elliptic, shortly petiolate; corolla bright salmon-pink, tube 22–25 mm long ..... 15. *S. hallaei*
5′. Calyx lobes about as long as tube; leaf lanceolate, sessile; corolla mauve, tube 12–13 mm long ..... 2. *S. angolensis*
6(4). Plant succulent, usually branched from base (tufted); leaf reduced, ≤10 mm long, appressed 7
6′. Plant not succulent, unbranched or if branched then from middle; leaf large, > 10 mm long, ascending or spreading 9
7(6). Corolla densely pubescent, bent proximally within calyx teeth; lower corolla lobes lanceolate ..... 21. *S. lepidagathidis*
7′. Corolla sparsely pubescent or glandular-pubescent, bent distally above calyx; lower corolla lobes obovate 8
8(7). Plant glabrate, about 6 cm tall, perennial; corolla tube up to 8 mm long ..... 12. *S. gastonii*
8′. Plant sparsely to densely hispid or pubescent, > 10 cm tall, annual (except strain on *Euphorbia*); corolla tube 8–14 mm long ..... 13. *S. gesnerioides*
9(6). Calyx lobes deltate, < ½ the tube length, adaxial lobe reduced to < 1 mm 10
9′. Calyx lobes linear to lanceolate, > ½ the tube length, adaxial lobes > 1 mm 12
10(9). Herbage with dense retrorse hispid hairs; corolla retrorsely pubescent; leaf 1–2 mm wide ..... 28. *S. yemenica*
10′. Herbage with dense ascending hispid hairs; corolla with ascending hairs; leaf >2 mm wide 11
11(10). Plant branched; corolla > 10 mm long, lobes of lower lip 7–15 mm long, 3–6 mm wide; calyx tube 5–10 mm long ... 16. *S. hermonthica*
11′. Plant usually unbranched; corolla < 10 mm long, lobes of lower lip 1–3 mm long, 1–1.5 mm wide; calyx tube 3–4 mm long ..... 14. *S. gracillima*
12(9). Corolla white (rarely pink or yellow); upper bracts as long as or longer than calyx; flowers sessile, spike longer than vegetative stem ..... 24. *S. passargei*
12′. Corolla pinkish purple; upper bracts shorter than calyx; flowers shortly pedicellate, raceme shorter than vegetative stem 13
13(12). Calyx tube 4–6 mm long; lower corolla lobes 4–8 mm long, 2–5 mm wide ..... 5. *S. aspera*
13′. Calyx tube 1.5–3 mm long; lower corolla lobes 2–3 mm long, 1–2 mm wide ..... 7. *S. brachycalyx*
14(1). Leaf pinnatifid; flowers solitary, few, axillary ..... 25. *S. pinnatifida*
14′. Leaf not pinnatifid; flowers in terminal raceme or spike 15
15(14). Leaf > 3 mm wide, with 2 to 5 prominent veins, margin toothed 16
15′. Leaf < 3 mm wide, veins obscure, margin entire 20
16(15). Corolla salmon-pink; lower bracts > 2 cm long; inflorescence lax at base 17
16′. Corolla pink or white; lower bracts < 2 cm long; inflorescence compact 18
17(16). Stem hairs stiff; lower bracts distinctly 3-veined, toothed; lower corolla lobes 6–9 mm long ..... 11. *S. forbesii*

17′. Stem hairs soft; lower bract veins obscure, entire; lower corolla lobes 10–15 mm long ..... 20. *S. latericea*
18(16). Leaf linear, base of hairs inconspicuous; bracts linear ..... 9. *S. dalzielii*
18′. Leaf elliptic or narrowly elliptic, base of hairs enlarged; bracts lanceolate 19
19(18). Corolla pink, tube 10–13 mm long; lower corolla lobes 4–7 mm long, 2–4 mm wide; leaf shorter than internode ..... 19. *S. klingii*
19′. Corolla white, tube 17–24 mm long; lower corolla lobes 7–10 mm long, 4–8 mm wide; leaf longer than internode ..... 23. *S. macrantha*
20(15). Corolla brilliant scarlet with yellow throat; flowers opposite in dense raceme ..... 10. *S. elegans*
20′. Corolla not brilliant scarlet; flowers alternate in lax raceme or spike 21
21(20). Corolla white or creamy white 22
21′. Corolla pink, purple, yellow, or dark red 23
22(21). Calyx 8–12 mm long; corolla tube 10–15 mm long, lower lobes 4–8 mm long, 2–4 mm wide ..... 3. *S. angustifolia*
22′. Calyx 13–21 mm long; corolla tube 27–35 mm long, lower lobes 14–20 mm long, 10–17 mm wide ..... 27. *S. pubiflora*
23(21). Calyx with at least 15 ribs; plant perennial 24
23′. Calyx with 10 ribs; plant annual 25
24(23). Corolla purple; leaf linear, ≥ 1 cm long; calyx 11–15 mm long, lobes 5, equal ..... 18. *S. junodii*
24′. Corolla yellow; leaf lanceolate, < 1 cm long; calyx 8–9 mm long, lobes 5, unequal ..... 26. *S. primuloides*
25(23). Leaf ≥ 15 mm long, longer than internode; lower bracts > 15 mm long ..... 4. *S. asiatica*
25′. Leaf < 15 mm long, shorter than internodes; lower bracts < 15 mm long 26
26(25). Corolla tube 20–25 mm long; calyx 8–15 mm long ..... 8. *S. chrysantha*
26′. Corolla tube < 15 mm long; calyx up to 6 mm long 27
27(26). Plant 6 cm tall (rarely 20); lower surface of leaf and bract with a single row of hispid hairs along margin and midrib; calyx lobes about equaling tube length ..... 17. *S. hirsuta*
27′. Plant up to 40 cm tall; lower surface of leaf and bract with two rows of hispid hairs along margin and midrib; calyx lobes ½ the tube length ..... 22. *S. lutea*

1. Striga aequinoctialis A. Chev. ex Hutch. & Dalz., Expl. Bot. Afr. Oc. Fr. 1: 476. 1920. TYPE: Guinea (Conakry). Montagne de Nzo, *Chevalier 21035* (holotype, P!).

Perennial, to 50 cm tall, slender, glabrate, usually simple or with 2 or 3 branches from below middle, stem winged. Leaves 10–24 × 1(–2) mm, filiform, sessile, decussate, ascending, shorter than internodes, hispid along margins, margin entire, veins obscure. Bracts 4–6(–12) × 1(–2) mm, linear, hispid along margins, incurved, longer than calyx. Flowers decussate in open spikes, spike shorter than vegetative stem. Calyx 5-ribbed, 3–4 mm long; tube 2–2.5 mm long; lobes 5, unequal, lanceolate, 1–2 mm long, slightly shorter than tube. Corolla pale mauve, tube 10–12 mm long, bent and expanded distally above calyx, retrorsely pubescent; lobes of lower lip 3–7 × 2–3 mm, ovate, tip acute, upper lip 3–5 × 4–7 mm, slightly notched, tip obtuse.

Restricted to the moist montane grasslands of west tropical Africa between 5°16′N and 11°0′N (Fig. 3). Known from Guinea, Sierra Leone, Liberia, and Ivory Coast with only one collection from outside West Africa, *Young 1300* (MO), from Angola. Like *S. bilabiata*, *S. aequinoctialis* has a bilabiate corolla, decussate leaves, slender stem, retrorsely pubescent corolla and shares part of the geographical range. *Striga aequinoctialis* is distinguished by its glabrous stem, filiform leaves, and small calyces. The calyx is the smallest in the genus.

Selected specimens examined. ANGOLA. **Lunda South:** Saurimo-Dala Road, 11°2′S, 20°12′E, *Young 1300* (MO). GUINEA. **Eastern:** Kankan, 45 mi. NW of Beyla, 9°11′N, 9°9′W, *Collenette 3* (K). **Southern:** N'Zerekore, 7°43′N, 8°50′W, *Adam 5111, 24653, 24386, 26054, 26531* (all MO). **Western:** Fouta Djalon, near Bandakure, SW of Pita, 11°0′N, 12°16′W, *Adames 284* (K). IVORY COAST. **Northwestern:** Odienne, Tienko, 10°5′N, 7°30′W, *Morton & Gledhill 18771* (K). **Southwestern:** Danane, Nimba Mountains, 7°34′N, 8°24′W, *Boughey 18052, 18067b, 180995* (all K). LIBERIA. **Nimba:** Mountain Nimba, 7°34′N, 8°40′W, *Adam 21457* (K, MO, UPS), *Vallah 65* (K); Grassfield-Nimba-Ideria, 6°45′N, 8°30′W, *Adam 25550* (K, MO). **Sinoe:** Jedepo, 5°16′N, 8°20′W, *Adam 24039, 27478* (MO). **Liberia:** *Adam 25592* (K). SIERRA LEONE. Bintinani, *Morton & Gledhill 1093* (K). **Eastern:** Sankanbiaiwa, 8°45′N, 11°0′W, *Cole 199* (K). **Northern:** Bumbuna to Farangbaia, 9°1′N, 11°44′W, *Deighton 5146* (K); Loma, 9°15′N, 11°2′W, *Anonymous 9783* (K). **Western:** Freetown, Mendi (Njamumu), 8°29′N, 13°0′W, *Burbridge 510* (K).

2. Striga angolensis Mohamed & Musselman, Brittonia 49: 118–121. 1997. TYPE: Angola. Vila Luzo, R. Luena, *Young 1365* (holotype, MO!). Figure 1.

Perennial, to 70 cm tall, stiffly erect, simple or with 2 or 3 branches from below middle, densely glandular-pubescent, stem obtusely square. Leaves (10–)20–35 × 2–8 mm, lanceolate, sessile, alternate, coarsely toothed, 3-veined, longer than internodes at the lower part of stem, becoming shorter above. Bracts 5–8 × 2 mm, abruptly different from leaves, lanceolate, shorter than calyx. Flowers opposite, lax at the base of inflorescence, dense toward the apex, spike shorter than vegetative stem. Calyx 4- or 5-ribbed, 6–8 mm long; tube 2.5–4 mm long; lobes 4 or 5, subequal, lanceolate, 3–5 mm

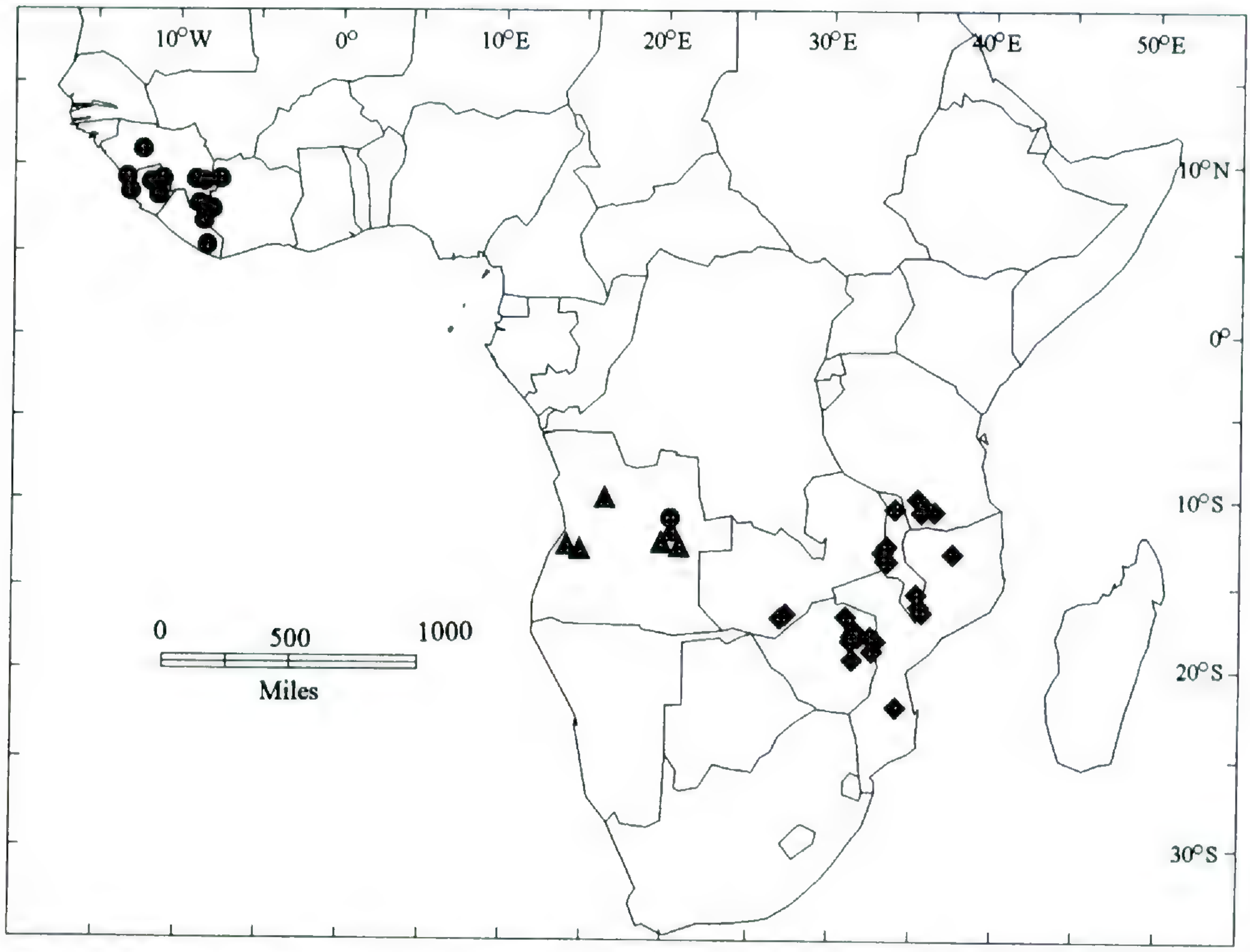


Figure 3. Distribution based on specimens of *Striga aequinoctialis* (circle), *S. angolensis* (triangle), and *S. angustifolia* (diamond).

long, as long as tube. Corolla weakly bilabiate, mauve, tube 12–13 mm long, bent and expanded distally above calyx, densely glandular-pubescent; lobes of lower lip 7–8 × 2 mm, obovate, upper lip 5 × 3 mm, obovate, slightly notched.

Striga angolensis is restricted to boggy grassland and wet areas in Angola between 9°48′S and 12°50′S (Fig. 3). Because there are numerous collections, we assume that *S. angolensis* was fairly common in this region, but we are not aware of any recent collections.

While many witchweeds are plants of dry areas, *S. angolensis* is part of a guild of witchweeds that inhabit wetlands, including *S. latericea*, *S. forbesii*, and *S. junodii* Schinz.

A statistical analysis of the genus (Mohamed, 1994) suggested that *Striga angolensis* is closely related to *S. hallaei* A. Raynal, *S. dalzielii* Hutch., *S. klingii* (Engl.) Skan, and *S. macrantha* (Benth.) Benth. They can be distinguished, however, on leaf and bract characters. *Striga angolensis* and *S. hallaei* are the only two *Pentapleurae* with prominently veined leaves, a feature common in at least six species in section *Polypleurae*. *Striga dalzielii*, *S. macrantha*, and *S. klingii* (sect. *Polypleurae*) differ from *S. angolensis* in having dense leafy spikes and leaf-like bracts that surmount the calyx, at least at the base of the inflorescence.

Selected specimens examined. ANGOLA. **Benguella:** Benguella, 12°36′S, 13°20′E, *Gossweller 3504* (K); Catumbela, 12°50′S, 14°40′E, *Faulkner A376* (K). **Lunda South:** small river between River Luachi & River Lumeji, 10°10′S, 20°12′E, *Young 1261* (ODU). **Malange:** Gando, 9°48′S, 16°10′E, *Faulkner A376* (K). **Moxico:** boggy grassland by River Chibamba, 12°40′S, 20°45′E, *Milne-Redhead 4161* (K); River Luena, Vila Luzo, 11°45′S, 19°50′E, *Exell & Mendonca 1353* (BM), *Young 1347* (BM), *Young 1365* (MO).

3. **Striga angustifolia** (Don) Saldanha, Bull. Bot. Surv. India 5: 70. 1963. *Buchnera angustifolia* Don, Prodr. Fl. Nepal: 91. 1825. TYPE: Nepal. *Wallich s.n.* (holotype, K!).

Annual, 10–50 cm tall, stiffly erect, unbranched or with 2 or 3 branches from below middle, pubescent or sometimes densely hispid with short upward curved hairs, stem obtusely square. Leaves 10–20(–30) × 1–3 mm, linear, alternate, as long as internodes, entire, veins obscure. Lower bracts 10–25 × 1–2 mm, leaf-like, longer than calyx, upper bracts subulate, shorter than calyx. Flowers alternate in lax racemes, raceme shorter than vegetative stem. Calyx 15-ribbed; ribs prominent, pilose, 8–

12 mm long; tube 3–6 mm long; lobes 5, equal, linear to lanceolate, 3–6 mm long, equal or subequal to the tube. Corolla creamy white with greenish tube, tube 10–15 mm long, bent and expanded distally above calyx, densely pubescent; lobes of lower lip 4–8 × 2–4 mm, obovate, upper lip 3–4 × 3–4 mm, broadly obovate, emarginate or truncate.

In Africa, *S. angustifolia* grows in the seasonally wet grasslands of Tanzania, Malawi, Mozambique, Zambia, and Zimbabwe between 9°52′S and 22°05′S (Fig. 3). It does not attack crops in eastern Africa, although it has been cited as a pest of cereals and sugarcane in India (Ramaiah et al., 1983). The occurrence of *S. angustifolia* in native grasslands and its absence in agroecosystems suggest it is indigenous to East Africa. *Striga angustifolia* is sympatric with and apparently related to *S. pubiflora* Klotzsch. and *S. junodii* (Mohamed, 1994). The Indian species *S. masuria* superficially resembles *S. angustifolia*, but the relationship between these two species has not been investigated. *Striga angustifolia* is widespread in India and Sri Lanka, and is known from a single collection in Oman. See Hepper (1986) regarding typification.

Selected specimens examined. MALAWI. **Central:** 6 km N of Kasungu, 12°55′S, 33°29′E, *Grosvenor & Renz 1038* (K); Kasungu Hills, 13°0′S, 33°15′E, *Jackson 2282* (K); Kasungu National Park, 13°35′S, 33°32′E, *Pawek 12015A* (K); near Tamanda Mission, *Robson 1094* (BM, K). **Northern:** Nyika Plateau, 10°30′S, 34°0′E, *McClounie 178* (K). **Southern:** Mount Mlanje above Likhubula, 16°5′S, 35°40′E, *Hepper 7368* (K); Saturi Stream, Likhubula Valley, Mulanj Mountain, 16°15′S, 35°27′E, *Chapman & E. G. 7186, 9000* (K); Zomba Mountain, Chivunde Valley, 15°30′S, 35°19′E, *La Croiz 2735* (MO); Zomba, 15°30′S, 35°19′E, *Hepper 7333* (K). MOZAMBIQUE. **Inhambane:** Mabote, 22°5′S, 34°5′E, *de Koning 7703* (K). **Niassa:** Marrupa, 13°8′S, 37°30′E, *Nuvunga 471* (K). TANZANIA. **Ruvuma:** just E Songea District Boundary, 10°42′S, 35°37′E, *Milne-Redhead & Taylor 10577* (K); Songea, 3 km W of Gumbiro, 10°15′S, 35°42′E, *Milne-Redhead & Taylor 10024* (K); S of Hanga River, 64 km from Songea on Njombe Road, also collected 120 km from Songea on Lindi road, Songea District, 9°52′S, 35°22′E, *Gilbert 17889* (K). ZAMBIA. **Southern:** 17 mi. NE of Choma, Brachystegia Wood, 16°40′S, 27°18′E, *Hutchison & Gilbert 3537* (K); Choma, 16°55′S, 26°59′E, *Robinson 1214* (K). ZIMBABWE. **Manicaland:** Inyanga, Cheshire, 18°15′S, 32°48′E, *Norlindh & Weimarck 4878* (K); Inyanga, Ghikori Reserve, 18°0′S, 32°30′E, *Masierson 217* (K); Umtali, Zimunya Reserve, 18°50′S, 32°37′E, *Chase 6854* (K). **Mashonaland North:** River Ruya, 16°50′S, 31°20′E, *Philcox & Drummond 9054* (K). **Mashonaland South:** Goromonzi, Kopje Domboshawa, 17°40′S, 31°34′E, *Philcox et al. 9093* (K); Goromonzi, Kopje Ngomakurira, 17°58′S, 31°22′E, *Philcox et al. 9127* (K); Lower Sabi, Videw, 19°20′S, 31°25′E, *Videw 8110* (K); Macheke, 18°0′S, 31°47′E, *Eyles 8799* (K); Marandellas, Charter Estate, 18°15′S, 31°20′E, *Cleghorn 2357* (K).

4. Striga asiatica (L.) Kuntze, Rev. Gen. Pl. 2: 466. 1891. *Buchnera asiatica* L., Sp. Pl.: 630. 1753. TYPE: Comoro Islands. St. Joanna, *Toreen s.n.* (LINN, photograph!).

Plant annual, up to 40 cm tall, stiffly erect, usually branched from middle, scabrous, hispid, stem obtusely square. Leaves (10–)20–50 × 1–3 mm, linear or narrowly elliptic, opposite, longer than internodes, margin entire, veins obscure. Lower bracts 15–35(–50) × 1–3 mm, leaf-like, upper bracts lanceolate, all bracts longer than calyx. Flowers alternate in lax terminal racemes, raceme longer than vegetative stem. Calyx 10-ribbed, 7–9 mm long; tube 5–7 mm long; lobes 5, equal, or 6 to 8 unequal, narrowly lanceolate, < ½ tube length. Corolla red with yellow throat, occasionally yellow, tube 11–14 mm long, bent and expanded distally above calyx, sparsely to densely pubescent; lobes of lower lip 3–5 × 2–3 mm, obovate, spreading, upper lip 3 × 3–4(–7) mm, emarginate, wider than long.

The red-flowered witchweed, which severely damages sorghum [*Sorghum bicolor* (L.) Moench.], maize (*Zea mays* L.), and other crops, is *S. asiatica.*

Hepper (1974) retained the name *S. asiatica* for the well-branched plant with dark-colored flowers over *S. lutea,* the name used for many years for the species of agronomic importance (e.g., in the United States, Sand et al., 1990). *Striga lutea* (see discussion below) and *S. hirsuta* may also have red corollas.

Striga lutea and *S. hirsuta* can also attack crops, but with no discernible damage. Because of confusion among these plants, reports in the literature of a red-flowered witchweed damaging a crop need to be verified.

Some workers, e.g., Musselman and Hepper (1986), *have* lumped *S. asiatica, S. lutea,* and *S. hirsuta* as a single species, *S. asiatica.* However, Mohamed (1994) reported consistent morphological, ecological, and phenological differences among these and concluded that three taxa should indeed be recognized.

The geographical range of *S. asiatica* includes southern and central Africa, where it is common on light sandy soils. It has also been collected from Abu Zaabal, Nile Delta, Egypt (Fig. 4), no doubt through contaminated grain seed, which is a major source of dispersal (Berner et al., 1992). In Madagascar and southern Africa, especially Botswana, *S. asiatica* does most damage to crops like sor-

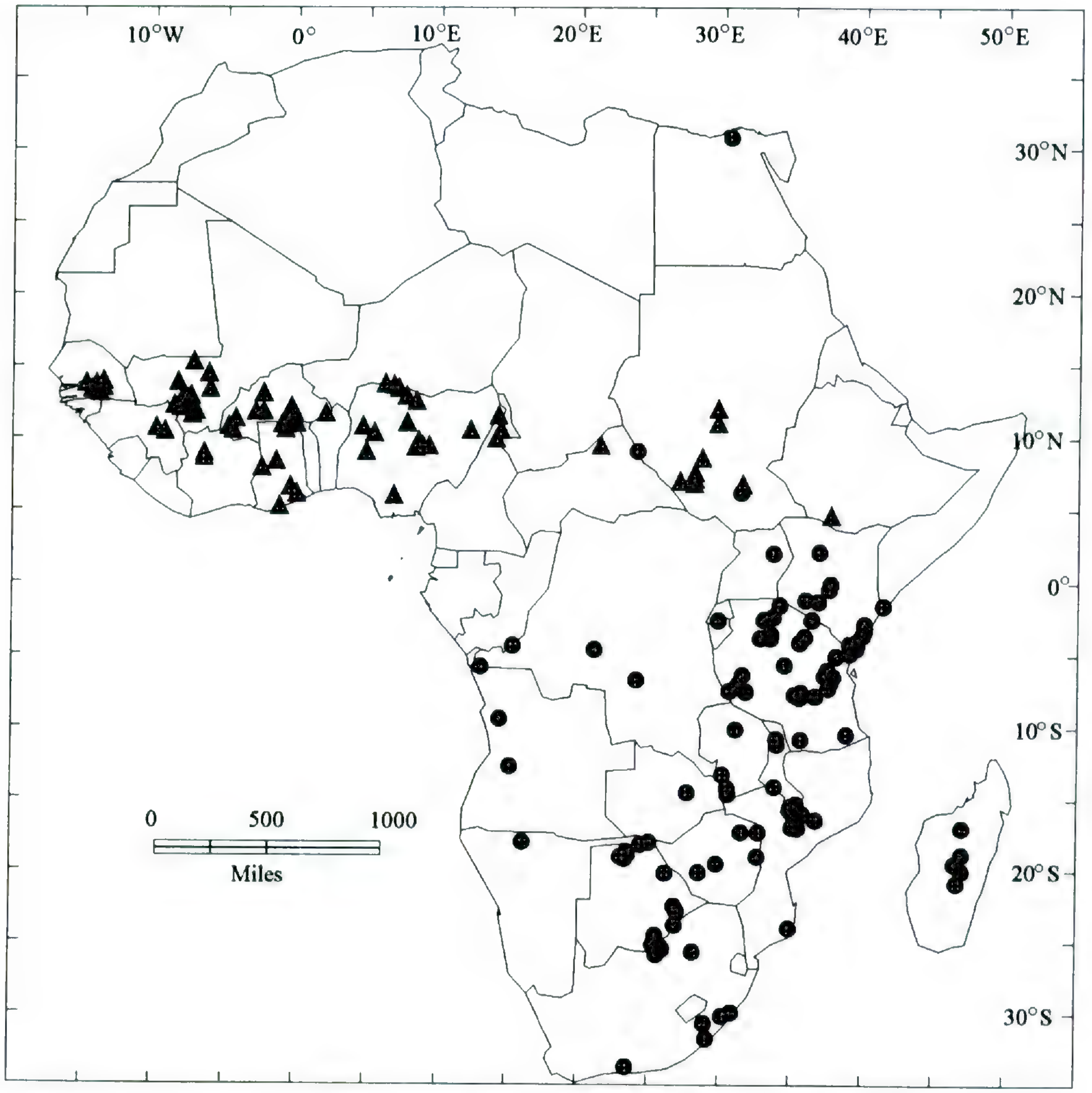


Figure 4. Distribution based on specimens of *Striga asiatica* (circle) and *S. aspera* (triangle).

ghum, maize, and millet [*Pennisetum americanum* (L.) Schumann] (Ralston et al., 1987).

Striga asiatica is not recorded as a pest in Africa outside this region except in Kenya (Musselman, unpublished) and its introduction in the Republic of Togo (Agbobli, 1991). Recently, rice in Madagascar has been attacked by *S. asiatica* (Geiger et al., 1996).

The center of origin of *S. asiatica* is most likely African (Raynal-Roques, 1991); it probably evolved in southern Africa, Madagascar, or the Mascarenes. This assumption is supported by the presence of the very similar and putatively related (Mohamed, 1994) *S. elegans* Benth. only in southern and eastern Africa. If correct, then the *S. asiatica* population introduced to the Carolinas in the United States came from southern Africa. Allozyme studies indicate that the introduction occurred once from an autogamous population (Werth et al., 1984).

Selected specimens examined. ANGOLA. **Cuanza North:** Cassoalala, 9°25′S, 14°20′E, *Anonymous 8347* (K). **Huambo:** 12°44′S, 15°5′E, *Wellman 1769* (K). **Loanda:** Zenzado, 8°48′S, 13°14′E, *Gossweller 4466* (K). BOTSWANA. Central Mahalatswe Lands 200 km N of Gabarone, *Musselman 7003* (ODU). **Central:** Swooning, near Serowe, 22°28′S, 26°47′E, *Mitchison 1975* (K). **Chobe:** Formabachena, near Pandamatenga, 18°9′S, 24°24′E, *Musselman et al. 9/4/86* (ODU). **Ngamiland:** Okavango, Moremi Wildlife, 19°10′S, 23°16′E, *Smith 845* (K). **Southern:** Lobatsi, Magabane, 25°12′S, 25°38′E, *De Bell 878* (K). BURUNDI. **Eastern:** Astrida (Rubona Ineac), 2°33′S, 29°45′E, *Michel 5367* (K). CONGO. **Kinshasa:** Kinshasa (Leopoldville), 4°20′S, 15°15′E, *Baldwin s.n.* (K). DEMOCRATIC REPUBLIC OF CONGO. **Bas-Zaire:** Boma, 5°48′S, 13°0′E, *Soyaux 189* (K). **Kasai Oriental:** Gandajika, 6°45′S, 23°57′E, *Sieber 2401* (GH). EGYPT. **Delta:** Abu Zaabal, 30°15′N, 31°21′E, *Elhadi & Khattab 90/188* (S). KENYA. **Coast:** Kilifi, 3°35′S, 39°55′E, *Moggridge 548* (K). **Eastern:** Jakake Forest, *Jeffery K242* (K). **Nairobi:** Nairobi, 1°17′S, 36°48′E, *Napier 51* (K). **North Eastern:** Chom Airship, 18 mi. NW Kiunga, 1°35′S, 41°22′E, *Gillespie 186* (K). **Rift Valley:** Isiolo to Mathews

Range, 0°24′N, 37°33′E, and Mountain Nyiru, 2°10′N, 36°50′E, *Nesbould 1958* (K). LESOTHO. **Western:** Mafetieng, 29°50′S, 27°10′E, *F.B. 6431* (K). MADAGASCAR. **Southern:** Katoala, S of Ambovombe, 25°8′S, 46°5′E, *Decary 9014* (US). MALAWI. **Central:** Bunda Agriculture College Farm, 23 km S of Lilongwe, 14°8′S, 33°47′E, *Brummit 8661* (K). **Northern:** Rumpi, 1 mi. up Shelinda River, 10°55′S, 33°53′E, *Pawek 12566* (K). **Southern:** Lower Shire Valley, 15°45′S, 35°0′E, *Lawrence 15* (K). MOZAMBIQUE. **Cabo Delgado:** 15 km on Mocimboa–Mueda Road, 10°40′S, 40°35′E, *Jasen 8158* (K). **Gaza:** 3 km from Chibuto, 24°39′S, 33°30′E, *De Lemes & Balsinhas 92* (K). **Inhambane:** Govuro, 7 km from Banamana, 24°2′S, 34°54′E, *Correia & Marques 4142* (K). **Manica:** Odzi River Valley, 18°59′S, 32°37′E, *Jeagu 84* (K). **Maputo**: Namaacha, 25°59′S, 32°05′E, *Sousa 555* (K). **Niassa:** E Coast of Lake Nyasa, *Johnson 101* (K). **Zambezia:** Lugela, Hamagru, 16°25′S, 36°43′E, *Faulkner 90* (K). NAMIBIA. **Owambo:** Olukonda 18°0′S, 16°3′E, *Reed 3/1888* (K). SOUTH AFRICA. **Natal:** Durban North, Prospect, 29°55′S, 30°56′E, *Forbes 1/1929* (K). **Nxikazi:** Kangwane Homeland, *Musselman 7022* (ODU). **Orange Free State:** Kroonstad, 27°46′S, 27°12′E, *Rogers 96* (US). **Transki:** Grigualand East, Clydis-dall, 30°40′S, 29°0′E, *Tyson 1222* (GH). **Transvaal:** near Pretoria, 25°40′S, 28°10′E, *Gkvile 41* (K). SUDAN. **Al-jazirah:** Gir, *Anonymous 2159* (K). **Bahr Al-Gabal:** Addai, 9°50′N, 24°0′E, *Anonymous 2180* (K). **Northern:** Ghattas, *Schweinfurth 2285* (K). **Jungali:** Bor, Zandi Land, Li Atafu, 6°12′N, 31°20′E, *Wyld 225* (K). TANZANIA. **Arusha:** Endabash, Lake Manyara National Park, 3°40′S, 35°50′E, *Greenway & Polhill 11305* (K). **Dar es Salaam:** Dar es Salaam, 6°47′S, 39°18′E, *Kuntze* 7/iv94 (US). **Iringa:** Ruaha National Park, at road to Idodi, 7°40′S, 35°25′E, *Bjronstad 1294* (K). **Lindi:** Nachingwea, 10°28′S, 38°49′E, *Anderson 846* (K). **Mafia Island:** Kikuni, *Greenway 5097* (K). **Mara:** Kampi Ya Mawe, 25 mi. from Scronera Musoma, 1°30′S, 34°6′E, *Greenway 10519* (K). **Morogoro:** Nguru Mountains, 6°3′S, 37°26′E, *Thulin & Mhoro 3140* (K). **Mwanza:** Nyahanga, 2°20′S, 33°3′E, *Tanner 810* (K). **Pwani:** 23 km SE of Bagamoyo, 6°34′S, 39°1′E, *Thulin & Mhroro 488* (K). **Rukwa:** Tumba, 7°30′S, 31°45′E, *Bullock 3770* (K). **Ruvuma:** Tunduru, 60 mi. from Masasi, 11°7′S, 37°2′E, *Richards 17980* (K). **Shinyanga:** Shinyanga, 3°30′S, 33°30′E, *Choner 2005* (K). **Singinda:** Manyoni, S of Itigi Station, 5°37′S, 34°27′E, *Greenway & Polhill 11736* (K). **Tanga:** Dahali-Madanga, 4°45′S, 39°′E, *Tanner 3018* (K). **Zanzibar:** Chaani, and Pemba, *Greenway 1204* (K). UGANDA. **Eastern:** Amuria, 2°4′N, 33°37′E, *Williams 32* (K). ZAMBIA. **Central:** near Kapushi River, Lukanga Plains, 14°30′S, 27°40′E, *Troprell 2050* (K). **Eastern:** Luangwa Valley, Tunwe Munkanya, 14°2′S, 30°30′E, *Phiri 79* (K). **Northern:** Chisnoima Falls, Luombi River, 10°6′S, 31°0′E, *Richards 5230* (K). ZIMBABWE. **Mashonaland North:** Midlands, near Makava, 17°17′S, 32°42′E, *Musselman 4/17/86* (ODU). **Matabeleland North:** Bulawayo, Waterford, 20°7′S, 28°32′E, *Norrgrann 459* (K). **Midlands:** Gwelo, Msasa-Mnondo, Senka Area, 19°0′S, 29°46′E, *Biegel 1912* (K).

5. Striga aspera (Willd.) Benth., Companion Bot. Mag. 1: 362. 1835. *Euphrasia aspera* Willd., Syst. 2: 776. 1801. TYPE: Ghana. *Isert s.n.* (holotype, BM!; isotype, C not seen).

Striga aspera var. *schweinfurthii* Skan, Fl. Trop. Afr. 4(2): 403. 1906. TYPE: Sudan. *Schweinfurth 1992* (holotype, K!).

Striga aspera var. *filiformis* Benth., in DC. Prodr. 10: 501. 1846. TYPE: Niger. *Vogel 15* (holotype, K!).

Annual, to 53 cm tall, slender, unbranched or branched from the middle, densely hispid, stem obtusely square. Leaves (10–)20–35(–60) × 1(–2) mm, opposite, linear, ascending or spreading, longer than internodes, margin entire, veins obscure. Lower bracts 8–30(–40) mm, leaf-like, longer than calyx, upper bracts lanceolate, shorter than calyx. Flowers alternate in open racemes, raceme shorter than vegetative stem. Calyx 5-ribbed, 5–10 mm long; tube 4–6 mm long; lobes 5, almost equal, linear, 3–5 mm long, as long as tube. Corolla weakly bilabiate, pinkish purple (rarely white), tube 12–16 mm long, bent and expanded distally above calyx, sparsely glandular-pubescent; lobes of lower lip 4–8 × 2–5 mm, obovate, upper lip 3–7 × 4–7 mm, emarginate or subtruncate, as long as wide.

Superficially, *Striga hermonthica* and *S. aspera* resemble one another. There are three characters to distinguish between them: corolla tube bend, pubescence, and size of calyx lobes. In the field, *S. aspera* can be differentiated from *S. hermonthica* by its slender stem and the pinkish purple corolla tube bent distally above the calyx teeth. The tube of *S. hermonthica,* on the other hand, bends in the middle, that is, proximally within the calyx teeth. Unfortunately, there are plants with intermediate characters. *Striga aspera* is sparsely glandular-pubescent, while *S. hermonthica* lacks a glandular-pubescent corolla tube. The glandular pubescence of the corolla and the short calyx lobes are the most consistent characters separating these two species. Calyx lobes in *S. aspera* are almost equal to the tube length with the adaxial lobe more than 1 mm long, whereas the lobes of *S. hermonthica* are short, triangular. The adaxial lobe is less than 1 mm long in *S. hermonthica.*

Leaves of *S. hermonthica* are more than 2 mm wide, whereas leaves of *S. aspera* are rarely 2 mm wide. This and the smaller stems make the overall aspect of *S. aspera* more delicate.

Striga aspera and *S. hermonthica* interbreed; both are outbreeders. Aigbokhan et al. (1998, 2000) demonstrated a high rate of interfertility between the two species. Morphological differences between the two species, compared to within-species variation, and the low germination percentage of their hybrids' seeds (Aigbokhan et al., 1998), support the current taxonomic treatment of recognizing two species. Chromosome numbers are also

different, with *S. aspera* N = 18 and *S. hermonthica* N = 19 (Aigbokhan et al., 1998).

Striga aspera, in its more delicate forms, may resemble *S. brachycalyx* Skan and *S. passargei*, and the three species are sympatric in most of their range.

Striga aspera is in the Sahelian and Sudanian domains of Africa (Fig. 4), from Senegal to Sudan between 4°43′N and 15°18′N (Fig. 4). Together with *S. gesnerioides* and *S. hermonthica* it has the most northern distribution of all species. Unlike *S. hermonthica*, however, the range of *S. aspera* does not extend south of the equator. *Striga aspera* has been reported to attack maize but not sorghum in Ethiopia (Parker, 1988) and fonio [*Digitaria exilis* (Kippist) Stapf], maize, rice (*Oryza sativa* L. and other species), and sugarcane in West Africa (Parker & Riches, 1993). The extension of agriculture into natural grasslands may expose crops to this species, especially in West Africa where it is common.

Kenfack et al. (1996) have shown that *S. aspera* in northern Cameroon has the broadest host range (Poaceae and Cyperaceae, 18 genera, 24 species) of the eight species of witchweeds they studied.

Selected specimens examined. BURKINA FASO. **Center East:** 5 km N of Tenkodogo, 11°50′N, 0°19′W, *Safa & Musselman* 10/25/87 (ODU). **Eastern:** Fada N'Gourma, 12°12′N, 0°25′E, *Raynal 22127* (K). **Haut Bassins:** Banfora, 10°50′N, 4°50′W, *Raynal 22394, 22395* (K). **Northern:** 5 km E of Godi, 11°55′N, 2°24′E, *Safa & Musselman* 10/12/87 (ODU). **Volta:** Boromo, 11°50′N, 2°55′W, *Raynal 22309* (K). CAMEROON. **Northern:** Champs/Piedmont, Maroua, 10°35′N, 14°22′E, *Meurillon 1363* (K). CENTRAL AFRICAN REPUBLIC. **Vakaga:** Dongolo Plains, 9°33′N, 21°24′E, *Fay 2794* (K). ETHIOPIA. **Sidamo:** Upper Birr State Farm, N of Bore, 4°43′N, 37°40′E, *Parker 4065* (K). GAMBIA. **Central:** Denfi, *Terry 3173* (K). **Eastern:** Basse, 13°25′N, 14°28′W, *Terry 1942* (K). GHANA. **Eastern:** 2/3 of way between Adidame and Ho, 6°40′N, 0°30′E, *Morton s.n.* (K). **Northern:** just N of Buige Bridge on Kintampo to Tamale Road, 8°25′N, 1°30′W, *Hossain & Agyakwah 37826* (K). **Upper:** Bolgatanga, 10°40′N, 0°50′W, *Hossain & Agyakwah 37829* (K, US). GUINEA. **Eastern:** Kankan, 10°30′N, 9°20′W, *Pobeguin 1020* (K). IVORY COAST. **Central:** CIDT Office at Kouto, 45 km N of Bouandougou, 9°0′N, 6°32′W, *Riches 230, 233* (K). MALI. **Koulikoro:** 11 km N of Didieni, 13°55′N, 8°25′W, *Musselman & Mohamed* 10/6/88 (ODU). **Segou:** Famabougou, 17 km from Sokolo, 14°39′N, 6°15′W, *Raynal 22581* (K). NIGER. Boundom to Chetimari, *Saadou 1839* (K). **Dosso:** Dongondoutchi, 13°50′N, 4°8′E, *Parker 2314* (ODU). **Maradi:** Dan Issa Road, 3 mi. from Maradi, 13°30′N, 7°7′E, *Hall 452* (K). NIGERIA. **Bauchi:** Hassan Estate, Luna Road, 10°10′N, 9°20′E, *Knepper* 9/26/89 (ODU). **Kano:** 7 km S of Katsina on Kano Road (163 km N Kano), 12°57′N, 7°40′E, *Parker 2046* (K). **Plateau:** Jos Plateau, Naroguta, 9°50′N, 4°35′E, *Lawlor & Hall* n *46508* (K). **Sokoto:** 10 mi. N of Bussa, 50 mi. S of Yelwa, 10°15′N, 4°35′E, *Cook 358* (K). SENEGAL. **Oriental:** 41 km NE of Velingara, 13°26′N, 13°55′W, *Musselman 7085* (K, ODU). SUDAN. **Bahr Al-Ghazal:** on River Sue, between Peili and Wau, 7°10′N, 28°0′E, *Meyers 7329* (GH, K). **Jungali:** 12 km E of Nyany, 80 km N of Bor, 6°52′N, 31°25′E, *Lock 81/132* (K). **South Kurdufan:** Dilling, Nuba Mountains, 12°5′N, 29°41′E, *Andrews 6* (K). **Upper Nile:** Djur, Jeriba Ghaffas, 8°39′N, 28°35′E, *Schweinfurth 1992* (S).

6. **Striga bilabiata** (Thunb.) Kuntze, Rev. Gen. Pl. 3(2): 240. 1898. *Buchnera bilabiata* Thunb., Prodr. Pl. Cap 100. 1800. TYPE: South Africa. *Thunberg s.n.* (holotype, UPS!).

Perennial, 20–60 cm tall, stiffly erect, unbranched or with 2 to 3 branches from below middle, pilose or scabrous hispid. Stem either terete, square and furrowed, or winged. Leaves 2–6 × 1–2 mm, lanceolate, appressed, or 10–60 × 1–2(–5) mm, linear, ascending or spreading, shorter or longer than internodes, margin entire, veins obscure. Bracts 5–20 × 1–3 mm, linear or lanceolate, shorter or longer than calyx. Calyx tubular, 5-ribbed, 4–12 mm long; tube 2–6(–8) mm long; lobes 4 equal or 5 unequal, linear or deltate, 1–5 mm long. Corolla densely pubescent, tube 7–17 mm, bent and expanded proximally or distally; lobes of lower lip 1–4(–7) × 1–2 mm, upper lip 2–4(–7) × (1–)2–4(–7) mm, the three lobes of the lower lip and the two lobes of the upper lip are fused almost their entire length forming a strongly bilabiate corolla, tips obtuse or acute, short.

The lack of dependable characters separating *S. bilabiata*, *S. rowlandii* Engl., *S. barteri* Engl., *S. ledermannii* Pilger, and *S. welwitschii* Engl. led Hepper (1960) to reduce these species to subspecies of *S. bilabiata*. Statistical analysis of populations of each subspecies of *S. bilabiata* supported Hepper's treatment (Mohamed, 1994). All subspecies of *S. bilabiata* form a natural group with characters grading into one another. Like *S. bilabiata*, *S. linearifolia* also possesses a strongly bilabiate corolla, winged stem, perennial habit, and occurs in small populations. We consider *S. linearifolia* as a sixth subspecies of *S. bilabiata*; otherwise, we follow Hepper (1960).

The following is a key, followed by brief descriptions, of the subspecies of *S. bilabiata*:

KEY TO SUBSPECIES OF *S. BILABIATA*

1. Bract broader than leaf; lower corolla lobes obtuse 2
1′. Bract narrower than leaf, lower corolla lobes acute 3
2(1). Calyx lobes subulate, about same length as tube, flowers opposite in spikes, spike as long as vegetative stem ..... 6b. subsp. *bilabiata*
2′. Calyx lobes lanceolate, < ½ tube length, flow-

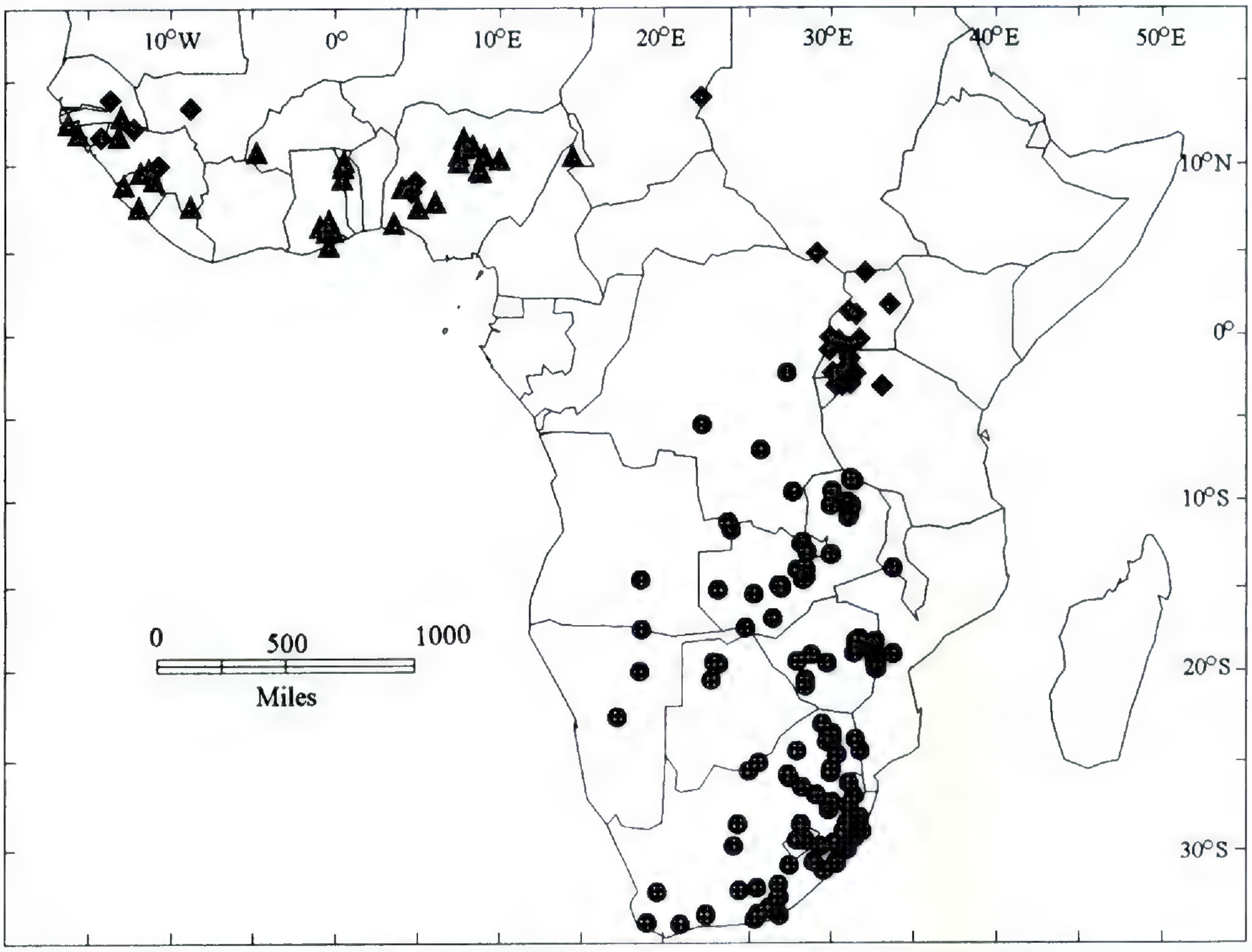


Figure 5. Distribution based on specimens of *Striga bilabiata* subsp. *barteri* (diamond), *S. bilabiata* subsp. *bilabiata* (circle), and *S. bilabiata* subsp. *rowlandii* (triangle).

ers imbricate in strobilus-like spikes, spike much shorter than vegetative stem ..... 6c. subsp. *jaegeri*

3(1). Leaf scale-like, < 1 cm long, plant covered with appressed, hispid hairs; corolla bent proximally within calyx teeth .... 6e. subsp. *linearifolia*

3′. Leaf not scale-like, 3–8 mm long; plant covered with soft or hispid divergent hairs; corolla bent distally above calyx 4

4(3). Plant ciliate; inflorescence strobilus-like; calyx 5 mm long ..... 6d. subsp. *ledermannii*

4′. Plant pilose; inflorescence not strobilus-like; calyx 6–12 mm long. 5

5(4). Stem winged; calyx lobes deltate, ½ tube length; spike shorter than vegetative stem ..... 6f. subsp. *rowlandii*

5′. Stem terete; calyx lobes linear, equaling tube length; spike longer than vegetative stem ..... 6a. subsp. *barteri*

6a. Striga bilabiata subsp. **barteri** (Engl.). Hepper, Kew Bull. 14: 414. 1960. *Striga barteri* Engl., Bot. Jahrb. Syst. 23: 514. 1897. TYPE: Nigeria. Nupe, *Barter 1170* (holotype, K!).

Striga glandulifera Engl., Bot. Jahrb. Syst. 23: 514, fig. H–J. 1897. TYPE: Sudan. *Schweinfurth 2931* (isotype, K!).

Plant to 18 cm tall, usually unbranched, densely pilose with divergent hairs, stem terete. Leaves 10–40 × 1–3 mm, linear, spreading, tortuous when dry, longer than internodes. Lower bracts 10–22(–45) × 1 mm, linear, longer than calyx, upper bracts lanceolate, shorter than calyx. Flowers alternate, the upper ones opposite, forming long open spikes, spike longer than vegetative stem. Calyx 6–12 mm long; tube 4(–8) mm long; lobes 5, unequal, linear, 3–4 mm long, about the same length as tube. Corolla lilac or pinkish, 12–14 mm long, bent and expanded distally above calyx, tube pubescent or glandular-pubescent; lobes of lower lip 2–3 × 1 mm, tips acute, upper lip 2–4 × 2 mm, tips acute.

The range of *S. bilabiata* subsp. *barteri* extends from Senegal to southern Sudan and south into Uganda and Tanzania between 14°N and 3°10′S (Fig. 5). Within this region, it occurs only in small scattered populations among grasses in rocky savanna woodland.

Selected specimens examined. BURUNDI. **Ruyigi:** Kigamba, 3°6′S, 30°33′E, *Reekmans 7125, 7166* (K). CHAD. **Logone-Oriental:** Doba, 8°39′N, 16°51′E, *Elliott s.n.* (K). GUINEA. **Northwest:** Kouroussa, Banko, 10°45′N, 9°54′W, *Adam 26896* (MO). GUINEA BISSAU. **Southern:** Madina do Boe, 11°45′N, 14°13′W, *Epirito Santo 3199* (K); Kipkarren, *Hill 755* (K). MALI. **Kayes:** Madina, 13°25′N, 8°48′W, *Robert 16608* (K). NIGER. *Barter 1170* (GH). NIGERIA. **Kwara:** slope near waterfall at head of Jebba Glen, 9°5′N, 4°50′E, *Meckle 1011* (K). RWANDA. **Southern:** Mutara, Ngarama, 2°20′S,

30°10′E, *Alcool 4116* (K). SENEGAL. **Oriental:** Tambacounda, Hasirik, 13°45′N, 13°40′W, *Adam 17917, 17934* (MO), *Tutin 162* (K). SUDAN. **Equatoria:** Zandi-land, Ibba, 4°50′N, 29°10′E, *Wyld 725* (BM). TANZANIA. **Mwanza:** Nyanhanga, Bugufi, Ngara, 3°10′S, 33°8′E, *Tanner S110* (K). **Ziwa Magharibi:** Bukoba, near Nddma, 1°30′S, 31°10′E, *Harrer 2227* (K); W Lake Province, Lusahanga, Biharamulo, 2°52′S, 31°12′E, *Tanner 5655* (K); W lake Prov., Murukurazo, Bugufi, Ngara, 2°25′S, 30°35′E, *Tanner 5108* (K). UGANDA **Eastern:** Sorati, Kapiri Ferry, 1°43′N, 33°35′E, *Lind 340* (K). **North Buganda:** Butemba-Singo, 1°8′N, 31°35′E, *Brown 2027* (K); Kambuga, Kizezi, 1°0′S, 29°58′E, *Purseglove 2711* (K). **Northern:** 2 km SE of Kikandwa, 3°38′N, 32°7′E, *Lester & Morrison 2337* (K). **South Buganda:** Masaka, 0°21′S, 31°45′E, *Elliott 1481* (K); Mubende, Lukwamnigo Hills, 1°0′N, 31°0′E, *Lanbrester 18* (K). **Western:** Mubende, 1°5′N, 31°5′E, *Lind 2027* (K), *Plant 706* (K); Toro, Oruha Hill, 0°25′N, 30°30′E, *Eggeling 4033* (K).

6b. **Striga bilabiata** Kuntze subsp. **bilabiata**

Striga thunbergii Benth., Companion Bot. Mag. 1: 363. 1835. TYPE: Botswana. *(?)Anonymous 2494* (holotype, K!).

Striga thunbergii var. *grandiflora* Benth., in DC. Prodr. 10: 502. 1846. TYPE: South Africa. Kat River Poort, *Drége 2297b* (holotype, K!).

Striga welwitschii Engl., Bot. Jahrb. Syst. 23: 514, 12, fig. F, G. 1897. TYPE: Angola. *Welwitsch 5821/5820* (holotype, K!; isotype, BM!).

Striga thunbergii var. *grandiflora* Engl., in Baum Kunene-Samb. Exped. 369. 1903. TYPE: Angola. Longamindung, *Baum 552* (holotype, K!).

Plant 20(–40) cm tall, usually unbranched or with 2 or 3 branches from base, scabrous, densely hispid with divergent hairs, stem square, furrowed. Leaves 10–20(–30) × 1(–2) mm, linear or lanceolate, ascending, decussate, longer than internodes. All bracts similar, 6–18 × 1–3 mm, lanceolate, ciliate, enclosing calyx, broader than leaves. Flowers opposite in short, dense spikes, spike as long as vegetative stem. Calyx 6–10 mm long; tube 3–5(–7) mm long; lobes 5, unequal, subulate, 3–5 mm long, about the same length as tube. Corolla white or mauve, tube 10–15 mm long, bent and expanded proximally within calyx teeth, densely glandular-pubescent; lobes of lower lip 2–4 × 1–2 mm, tips obtuse, upper lip 2–5(–7) × 2–4 mm, tips obtuse.

Striga bilabiata subsp. *bilabiata* is found only between 2°22′S and 34°02′S (Fig. 5) in southern Democratic Republic of Congo, Zambia, Angola, Zimbabwe, Botswana, and South Africa. It is less frequent to the north and unknown from the East African coast. This subspecies varies considerably in height but is typically about 20 cm tall. *Striga bilabiata* subsp. *bilabiata* is strongly autogamous (Ralston et al., 1987).

Selected specimens examined. ANGOLA. **Banguella:** Banguella, 12°36′S, 13°20′E, *Gossweller 2234* (K). **Moxico:** 2 mi. N of Jimele River bridge on Caianda–Mwinilunga road, 11°17′S, 23°48′E, *Richards 17147* (K). BOTSWANA. 3 mi. SW Nzinzi down Omuramba Mpungu, *Winter 3995* (K). **Ngamiland:** banks of Okavango River at Runtu, 19°34′S, 23°15′E, *Winter & Marais 4478, 4913* (K). **North District:** Tsetse Fly Camp, W of Parakarungu, *Lambrecht 264* (K). **Southern:** Digkatlong Ranch, 25°40′S, 25°3′E, *Hansen 3016* (K, MO). DEMOCRATIC REPUBLIC OF CONGO. **Kivu:** 6.3 km NW of Katshungu, 2°22′S, 27°22′E, *Anonymous 4863* (K). **Shaba:** Katanga, Biano Plateau, 7°0′S, 27°22′E, *Russell 41* (K). LESOTHO. **Central:** N of Blue Mountains Pass, 29°30′S, 28°30′E, *Dieterlen 527* (K). **Northern:** Leribe, 28°58′S, 28°0′E, *Dieterlen 529* (K). MALAWI. **Central:** Lilongwe, 13°59′S, 33°47′E, *Jackson 649* (K). MOZAMBIQUE. **Maputo:** Namaacha, Mt. Ponduine, 26°0′S, 32°25′E, *Schuter & Nuvunga 6644* (K). NAMIBIA. **Windhoek:** 20 mi. SW of Windhoek, Farm Haris, 22°35′S, 17°4′E, *Winter 2540* (K). SOUTH AFRICA. **Cape:** Stockenstrom, Nogsback, 32°40′S, 26°50′E, *Leighton 2948* (MO). **Natal:** Bethlehem, Royal National Park, Tugela River, 28°45′S, 29°28′E, *Ross 2240* (K). **Orange Free State:** Barkly West, Cristaalfrontein, 28°30′S, 24°26′E, *Acocks 1481* (K). **Transkei:** Griqualand East, Clydesdall, 30°40′S, 29°0′E, *Tyson 894* (GH). **Transvaal:** Albany, Alicedale Road, 26°6′S, 28°5′E, *Bayliss 8112* (MO, US). SWAZILAND. **Hhohho:** 5 km NW of Mbabane, 26°18′S, 31°6′E, *Kemp 701* (MO, US). **Matobo:** Farm Besua Kobila, *Miller 7260* (K). ZAMBIA. **Central:** 5 mi. from Mumbwa on Road to Kafue Hoek, 15°0′S, 26°52′E, *Drummond & Cookson 6185* (K). **Copperbelt:** 3 km NE of Mufulira, 12°30′S, 28°17′E, *Cruse 372* (K). **Lusaka:** Broken Hill, 14°30′S, 28°29′E, *Mutimushi 932* (K). **Mbala:** Lyopa Plain, *Sanane 980* (K). **Northern:** 16 km N of Mbala, 8°40′S, 31°15′E, *Philcox et al. 10170* (K). **Western:** 4 mi. N of Kalobo, 14°55′S, 22°30′E, *Drummond & Cookson 6451* (K). ZIMBABWE. Farm Besna Kobila, *Miller 2630, 2671, 5737* (K). **Lomagundi:** Great Dyke, *Philcox & Muller 9080* (K). **Manicaland**: Inyanga, Cheshire, 18°15′S, 32°42′E, *Norlindh & Weimarck 44347* (K). **Mashonaland South:** Macheke, 18°6′S, 31°47′E, *Eyles 2015* (K). **Matabeleland South:** Matopo, 20°28′S, 28°29′E, *Miller 1590* (MO).

6c. **Striga bilabiata** subsp. **jaegeri** Hepper, Kew Bull. 14: 415. 1960. TYPE: Mali. Massif de Kita, *Jaeger k.2 (753/51)* (holotype, K!).

Striga brouilletii Mielcarek, Bull. Jard. Bot. Belg. Bull. Nat. Plantentuin Belg. 58: 121–127. 1988. TYPE: Guinea (Conakry). Mt. Kankan: Koumbankourou, *Lisowski 80579* (holotype, POZG, photo!; isotype, BR not seen).

Plant 30–40 cm tall, unbranched, densely hispid with divergent hairs, stem winged. Leaves 40–70 × 2–3 mm, linear to narrowly elliptic, spreading, decussate, alternate toward the apex, with prominent midrib, longer than internodes. All bracts similar, 9–11 × 3–4 mm, widely obovate, acuminate, ciliate, enclosing calyx, broader than leaves. Flowers imbricate in short, dense, strobilus-like spikes, spike much shorter than vegetative stem. Calyx 7–8 mm long; tube 5–6 mm long, lobes 4 equal, or 5

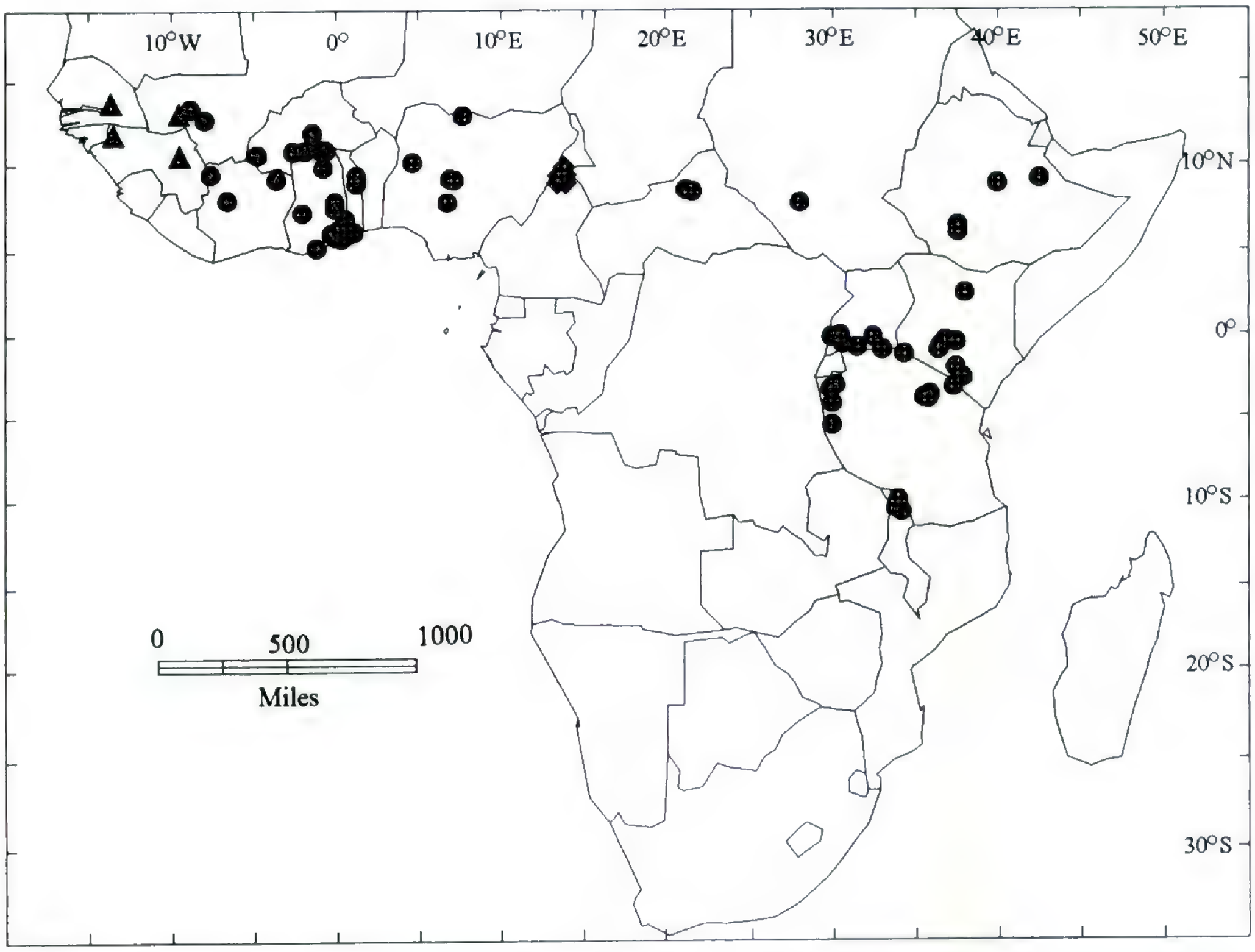


Figure 6. Distribution based on specimens of *Striga bilabiata* subsp. *jaegeri* (triangle), *S. bilabiata* subsp. *ledermannii* (diamond), and *S. bilabiata* subsp. *linearifolia* (circle).

unequal, lanceolate, 1–2 mm long, < ½ tube length. Corolla mauve, tube 15–17 mm long, bent and expanded distally above calyx, densely pubescent; lobes of lower lip 2–4 × 2 mm, tips obtuse, upper lip 1.5–2.5 × 1–2 mm, tips obtuse.

Striga bilabiata subsp. *jaegeri* is a rare taxon determined by its broad bracts and short compact spikes. It has been collected from savanna on rocky plateaus in Guinea, Senegal, and Mali between 10°18′N and 13°50′N (Fig. 6). *Striga bilabiata* subsp. *barteri* and *S. bilabiata* subsp. *jaegeri* have more northerly distributions than *S. bilabiata* subsp. *linearifolia* and *S. bilabiata* subsp. *rowlandii* (Figs. 5, 6).

Selected specimens examined. GUINEA. **Northern:** 5 km NW of Karifameriya, 11°55′N, 13°30′W, *Lisowski 80551* (BR). MALI. **Kayes:** Kita, 13°10′N, 9°34′N, *Jaeger 753/51, 2541* (K). SENEGAL. **Oriental:** Tambacounda, Gouloumbou, 14°0′N, 13°40′W, *Adam 14861* (MO).

6d. Striga bilabiata subsp. **ledermannii** (Pilger) Hepper, Kew Bull. 14: 413. 1960. *Striga ledermannii* Pilger, Bot. Jahrb. 45: 215. 1910. TYPE: Cameroon. Garoua, *Ledermann 4529* (lectotype, designated by Hepper (1960), BM!).

Plant 36 cm tall, unbranched, scabrous, with ciliate divergent hairs, stem winged. Leaves 10–23 × 2–3(–5) mm, lanceolate, decussate, decurrent, appressed, enclosing stem, longer than internodes. All bracts similar, 5–15 × 2–4 mm, imbricate, elliptic or lanceolate, ciliate, enclosing calyx. Flowers imbricate in short, dense, strobilus-like spikes, spike shorter than vegetative stem. Calyx 5 mm long; tube 2.5–3 mm long, lobes 5, unequal, linear, 2–3 mm long, about the same length as tube. Corolla pink, tube 12 mm long, bent and expanded distally above calyx, usually retrorsely pubescent; lobes of lower lip 1–2 × 0.5–1 mm, short, tips acute or obtuse, upper lip 3 × 1–2 mm, tips obtuse.

Striga bilabiata subsp. *ledermannii* is endemic to the northern province of Cameroon between 9°N and 10°N (Fig. 6). It is infrequent, like other subspecies of *S. bilabiata*, perhaps because it only parasitizes perennial hosts such as *Loudetia simplex* (Nees) C. E. Hubbard, *Schizachyrium sanguineum* (Retz.) Alston, and *Hyparrhenia* species (Kenfack et al., 1996). Although the hosts of other subspecies of *S. bilabiata* have not been studied, parasitism of perennial hosts may explain why *S. bilabiata* subsp. *barteri*, *S. bilabiata* subsp. *linearifolia*, and *S. bil-*

abiata subsp. *jaegeri* are infrequent and occur only in small populations.

Selected specimens examined. CAMEROON. **Northern:** 20 km SW of Dourbey, Biatonmi, *Fotius 2219* (K); 23 km S Garoua, 9°0′N, 13°25′E, *Musselman et al.* 10/16/88 (K, ODU); Bogalo by River Benue, 9°0′N, 13°50′E, *Talbot 313* (BM); Buar to Buala 9°34′N, 13°46′E, *Michael 9531* (K); Garoa, 9°16′N, 13°25′E, *Ledermann 4529* (BM); Jaiya Buge, 6°20′N, 16°15′E, *Michael 9706* (K); Kounden, 6°7′N, 14°38′E, *Saxer 254* (K); near Koubout, 9°57′N, 13°45′E, *Thomas 2452* (MO).

6e. Striga bilabiata subsp. **linearifolia** (Schum. & Thonn.) Mohamed, comb. nov. Basionym: *Buchnera linearifolia* Schum. & Thonn., Beskr. Guin. Pl.: 279. 1827. *Striga linearifolia* (Schum. & Thonn.) Hepper, Kew Bull. 14: 416. 1960. TYPE: Ghana. *Thonning 284* (holotype, C, holotype negative # 2785, 2786; isotypes, K!, P-JU not seen).

Striga strictissima Skan, Fl. Trop. Afr. 4(2): 407. 1906. TYPE: Nigeria. *Barter 1263* (holotype, K!).

Plant up to 62 cm tall, unbranched or with 1 to 2 branches from base, covered with dense, white, appressed, hispid hairs, stem winged. Leaves reduced, 3–8 × 1–2 mm, lanceolate, decussate, decurrent, appressed, shorter than internodes. All bracts similar, 5–7(–10) × 1–2 mm, lanceolate, slightly longer than calyx. Flowers opposite in dense spikes, spike shorter than vegetative stem. Calyx 4–7 mm long; tube 2–3(–4) mm long; lobes 5, unequal, linear, 1.5–3 mm long, about the same length as tube. Corolla whitish to bluish purple, tube 7–14 mm long, bent and expanded proximally within calyx teeth, densely retrorsely pubescent; lobes of lower lip 1–3(–7) × 0.5–1 mm, tips acute, upper lip 2–5 × 2–4 mm, slightly round.

Striga bilabiata subsp. *linearifolia* is widely distributed in Africa in wet grassy savannas. Its range extends from 13°N and reaches its southern limit in Malawi (10°55′S) (Fig. 6). Like other subspecies of *S. bilabiata*, it occurs in small populations often of only a few plants. For example, we gathered it once from populations of two plants in Mali, Ivory Coast, and Guinea (Conakry).

Selected specimens examined. ANGOLA. **Benguela:** Benguela, Sambo, 12°30′S, 13°26′E, *Teixerira 303* (BM). BURKINA FASO. **Central:** S of Sapones, 12°0′N, 1°30′W, *Safa & Musselman* 10/21/87 (ODU). **Haut Bassins:** 5 km S of Banfora, 10°40′N, 5°50′W, *Safa & Musselman*10/3/87 (ODU). BURUNDI. **Central:** Gitega, 3°20′S, 29°55′E, *Anonymous 1829* (K). **Southern:** Danga (Mosso), Bururi, 4°12′S, 30°0′E, *Reekmans 6786* (K). CENTRAL AFRICAN REPUBLIC. **Bangoran:** 4 km N of Camp Koumbala, Gaumba Creek, 8°31′N, 21°13′E, *Fay 7057* (MO). ETHIOPIA. **Gamu Gofa:** Gughe highlands, 6°3′N, 37°35′E, *Gilbert & Thulin 415* (MO). **Ogaden Harerge:** Harar, Harrar-Jijega, 9°13′N, 42°29′E, *de Wilde 6399* (MO). **Shewa:** Awash National Park, 8°55′N, 39°55′E, *Gilbert & S. B. 1250* (K). GHANA. **Accra:** Accra Plains, 5°35′N, 0°6′W, *Morton 6123* (K). **Eastern:** Keta Krachi, 5°55′N, 1°2′E, *Morton 7171* (K). **Northern:** 2 mi. S Nasia on Tamale-Bolgatanga Road, 9°55′N, 0°50′W, *Adams 4152* (K). **Upper:** 10 mi. from Lambusie, 10°52′N, 2°40′W, *Morton 8794* (K). **Volta:** Akuse, 6°6′N, 0°8′E, *Vigne 4359* (US). GUINEA. *Probegum 364* (K). IVORY COAST. **Northeast:** Gawi-seye, along Iringou River, 9°14′N, 3°41′W, *Amshoff 225* (MO). **Odienne:** Odienne, 9°31′N, 7°38′W, *Adam 27125* (MO). **Seguela:** Seguela and Man, 8°0′N, 6°38′W, *Ake 8755* (K). KENYA. **Central:** Lake Naivasha, 0°48′S, 36°20′E, *Mathew 6126* (K). **Eastern:** Embu, Rumbia, 0°32′S, 37°28′E, *Graham 2405* (K). **Rift Valley:** Chyulu Hills, 2°40′S, 37°52′E, *Gilbert & Kariuki 18863* (K). **Shiyanga:** Kahawa, Kenyatta College, 3°50′S, 32°36′E, *Akestrid 2527* (UPS). MALAWI. **Northern:** 27 mi. N of Rumpi, 10°55′S, 33°54′E, *Pawek 12317* (K, MO). MALI. **Koulikoro:** Simidij, 11°40′N, 7°35′W, *Musselman & Mohamed* 9/30/88 (ODU). NIGER. *Barter 1263* (GH). NIGERIA. **Kaduna:** Katsina, Maska, 13°0′N, 7°35′E, *Keay 25896* (K). **Niger:** between Gawu–Abuja Road Junction, 9°15′N, 6°50′E, *Onochie 18697* (K). **Northern:** Lemme, *Lely 146* (K). **Plateau:** Shere Mountains, 9°10′N, 9°50′E, *Hall & Daramola 67404* (MO). **Sokoto:** 10 km N of Bussa, 50 km S of Yelwa, 10°15′N, 4°35′E, *Cook 445* (K). SUDAN. **Bahr Al-Ghazal:** Wau, 7°46′N, 28°2′E, *Anonymous 1651* (K). TANZANIA. **Arusha:** 3 mi. E of Lake Manyara, 3°40′S, 35°53′E, *Lamprey 515* (K). **Kigoma:** Buha, 56 mi. from Kasulu on Kigoma Road, 5°30′S, 30°0′E, *Verdcourt 3330* (K). **Kilimanjaro:** E Kilimanjaro, Mran Rombo, 3°12′S, 37°20′E, *Haarer 517* (K). TOGO. **Central:** 9 km N of Sokode, 9°7′N, 1°10′E, *Hakki et al. 289* (K). UGANDA. **Bokora:** Iriri, *Nilson 332* (K). **Central:** E of Mengo, Kakonge, 0°15′N, 32°28′E, *Rwaburindore 580* (MO). **Eastern:** Busana, Bugisu, 1°15′N, 34°20′E, *Eggeling 350* (K). **South Buganda:** near Sanje Rest Camp, 0°49′S, 31°30′E, *Ankole & Eggeling 610* (K). **Southern:** Ankole, Ibanda, 0°8′S, 30°30′E, *Ragslane 1251* (US). **Western:** Busoga, 8 mi. W of Kamuli, 0°58′N, 33°1′E, *Wood 726* (K).

6f. Striga bilabiata subsp. **rowlandii** (Engl.) Hepper, Kew Bull. 14: 415. 1960. *Striga rowlandii* Engl., Bot. Jahrb. Syst. 23: 513. 1897. TYPE: Nigeria. Western Lagos, *Rowland 1893* (holotype, K!).

Plant up to 34 cm tall, unbranched or with 2 or 3 branches from base, sparsely to densely pilose with divergent hairs, stem winged. Leaves 10–55 × 1–3 mm, linear, decussate, decurrent, ascending, longer than internodes. Lower bracts 7–10(–18) × 1–2 mm, lanceolate, longer than calyx, upper bracts shorter than calyx. Flowers alternate at base of inflorescence, opposite above in a somewhat dense spike, spike shorter than vegetative stem. Calyx 6–9 mm long; tube 5–6(–8) mm long; lobes 5, unequal, deltate, 1–2 mm long, ½ tube length. Corolla pink, mauve, or white, tube 9–12(–15) mm

long, bent and expanded distally above calyx, densely pubescent; lobe of lower lip 2–4 × 1 mm, tips acute, upper lip 2–4 × 2–4 mm, tips acute.

The subspecies is endemic to the relatively wet, rocky-plateau grasslands in West Africa between 13°N and 6°10′N (Fig. 5); its range extends from Senegal to Cameroon.

Selected specimens examined. BURKINA FASO. **Haut Bassins:** 18 km N of Banfora, 10°39′N, 4°50′W, *Leeuwenberg 4357* (K, MO). CAMEROON. **Northern:** Mont Boulorb, 5 km N of Maroua, 10°37′N, 14°22′E, *Letouzey 624* (K). GAMBIA. *Hayes 539* (K). GHANA. **Eastern:** 2 mi. NE of Kwahu-Tofo on road to Adawso, 6°15′N, 0°15′W, *Innes 909* (K); 3 mi. N of Kwahu-Tafo, 6°17′N, 0°20′W, *Harris 195* (K). **Northern:** 10 mi. from Lumbuga, 9°20′N, 2°5′E, *Morton 8795* (K); 2 mi. beyond Kwaha-Tafo on Mankrong Road, 6°10′N, 0°18′W, *Morton A663* (K); Afram Glams, 6°50′N, 0°25′W, *Onsow 709* (K); Damongo, Yendi, 9°17′N, 0°22′E, *Hepper & Morton A3110* (K); Kwaha-Tafo, 6°10′N, 1°0′W, *Hall 131, 260* (K); Okroso to Otiso Ferry, 10°0′N, 0°25′E, *Hepper & Morton A3039* (K); Onyaboni to Jaketi, *Morton A3016* (K); Sesiamango, *Thomas D138* (K). GUINEA. **Northern:** Seriba, Gaoual, 11°50′N, 13°10′W, *Adam 14812* (MO). **Southern:** N'zerekore, 7°40′N, 8°50′W, *Adam 26898, 27661* (MO). IVORY COAST. **Northeast:** Dabakala, 45 km NW Dabakala, 8°32′N, 4°9′W, *Geerling & Bokdam 2029* (MO). NIGER. *Barter 1169* (GH). NIGERIA. **Bauchi:** Bauchi Plateau, 10°25′N, 9°55′E, *Lely 83* (K); 10°20′N, 9°45′E, *Lely P83* (K, MO). **Kaduna:** 3 mi. N of Zaria, 11°8′N, 7°43′E, *Milne-Redhead 5043* (K); Kaduna, 10°31′N, 7°25′E, *Hill 51* (K); near Zaria, 11°7′N, 7°43′E, *Dalziel 407* (K); Riruwai-Kano Hills, 11°15′N, 8°17′E, *Carpenter 192* (K); Zaria, Mando, Gayam, 10°27′N, 7°25′E, *Keay 25833* (K); Zaria, Samaru, 11°5′N, 7°43′E, *Harris 11* (K); N Nigeria, *Pobeguin 364* (K). **Oyo:** 8 mi. E of Igboho, Upper Ogun Forest Reserve, 8°47′N, 4°0′E, *Keay s.n.* (K); Kogosti, 27 mi. E of Ilesha, 7°35′N, 4°58′E, *Lowe 3378* (K). **Plateau:** Vom, Bauchi Plateau, 9°40′N, 8°48′E, *Young 185* (K). SENEGAL. **Casamance:** Ziguinchor, Bayotter, 12°35′N, 16°12′W, *Adam 13724* (MO). **Oriental:** Niokolo-Koba, 13°0′N, 13°0′W, *Berhaut 1630* (K); Tambacounda, Niokolo-Koba, 13°5′N, 13°0′W, *Adam 14200, 26891, 26897* (MO). SIERRA LEONE. Kafari (Malal), *Bakshi 146* (K); Lake Sonfon, *Gledhill 285* (K). **Northern:** Buyabuya Scarcies, 9°45′N, 12°10′W, *Elliott 4279* (GH); Falaba, 9°52′N, 11°20′W, *Elliott 5207* (K, MO); Loma Manza, 9°15′N, 11°2′W, *Thomas 155* (K); near Mongo, Bure Makonte Chiefdom, 9°40′N, 11°50′W, *Glanville 241* (K), *Adames 224* (K); Sumbuya, 7°39′N, 11°58′W, *Thomas 603* (K); Rhombe Swamp, *Adames 146* (K); Robis, *Jordan 190* (K).

In summary, we recognize six subspecies of *S. bilabiata*, the five subspecies originally proposed by Hepper (1960) in addition to *S. bilabiata* subsp. *linearifolia*. These form a natural group: they are generally autogamous, perennials, unbranched, have strongly bilabiate corollas, winged stems, and occur in small populations. Many of the morphological features used to distinguish other species, such as heights and sizes, overlap in this species complex, making them unreliable characters.

7. **Striga brachycalyx** Skan, Fl. Trop. Afr. 4(2): 403. 1906. TYPE: Nigeria. Nupe, *Barter 1858* (holotype, K!).

Striga warneckei Engl. ex Skan, Fl. Trop. Afr. 4(2): 414. 1906. TYPE: Togo. *Warnecke 201* (isotypes, BM!, K!).

Annual, to 70 cm tall, slender, branched from middle, scabrous, hispid, stem square, furrowed. Leaves (5–)20–37(–50) × 1(–2) mm, subopposite, linear, ascending or spreading, longer than internodes, margin entire, veins obscure. Lower bracts 5–20 × 1 mm, linear, as long as or longer than calyx, upper bracts subulate, shorter than calyx. Flowers alternate, forming somewhat open racemes, raceme shorter than the vegetative stem. Calyx 5-ribbed, 3–6 mm long; tube 1.5–3 mm long; lobes 5, almost equal, linear, 1–3.5 mm long, about the same length as tube. Corolla purple with white center, tube (6–)9–19 mm long, bent and expanded distally above calyx, sparsely glandular-pubescent; lobes of lower lip 2–3(–5) × 1–2 mm, tips obtuse, upper lip 1–2(–4) × 2–4 mm, obovate.

Striga brachycalyx is widespread and often common in dense stands in the savannas of west and central Africa between 13°12′N and 5°17′N (Fig. 7). Its range extends from Ivory Coast into Burkina Faso, Mali, Ghana, Togo, and Nigeria, reaching its eastern limits in Sudan. It is also known from Congo. This species is morphologically homogenous throughout its range. *Striga brachycalyx* resembles *S. aspera*, but can be distinguished by its smaller flowers and more slender stems. The two species are sympatric, found in relatively drier savannas, and frequently encountered in large populations. Unlike *S. aspera*, which occasionally invades agroecosystems, *S. brachycalyx* is not known to damage grains even though it is very common.

Selected specimens examined. BURKINA FASO. **Center East:** 5 km N of Tenkodogo, 12°0′N, 0°19′W, *Safa & Musselman* 10/9/87 (ODU). **Center West:** 5 km W of Godi, 11°55′N, 2°24′W, *Safa & Musselman* 10/12/87 (ODU). **Central:** 1 km S of Kamboiense, 12°25′N, 1°40′W, *Musselman 6118* (ODU). **Haut Bassins:** 5 km S of Banfora, 10°37′N, 4°44′W, *Safa & Musselman* 10/13/87 (ODU). **Northern:** 5 km N of Gourcy, 13°12′N, 2°22′W, *Safa & Musselman* 10/10/87 (ODU). **Volta:** near Boromo and Laba, 11°50′N, 2°55′W, *Raynal 2282* (K). DEMOCRATIC REPUBLIC OF CONGO. **Kasai Oriental:** Tumba Camp, Kasumanyense Mbuga, Rukwa Valley, 3°7′S, 23°34′E, *Siame 144A* (BM). GHANA. Ayafie, *Adams 4693* (K). **Northern:** Bole, 9°5′N, 2°29′W, *Morton A3295* (K). **Upper:** 4 mi. from Tono, Navrongo, 10°57′N, 1°40′W, *Irvine 4668* (K). IVORY COAST. **Northern:** Kor-

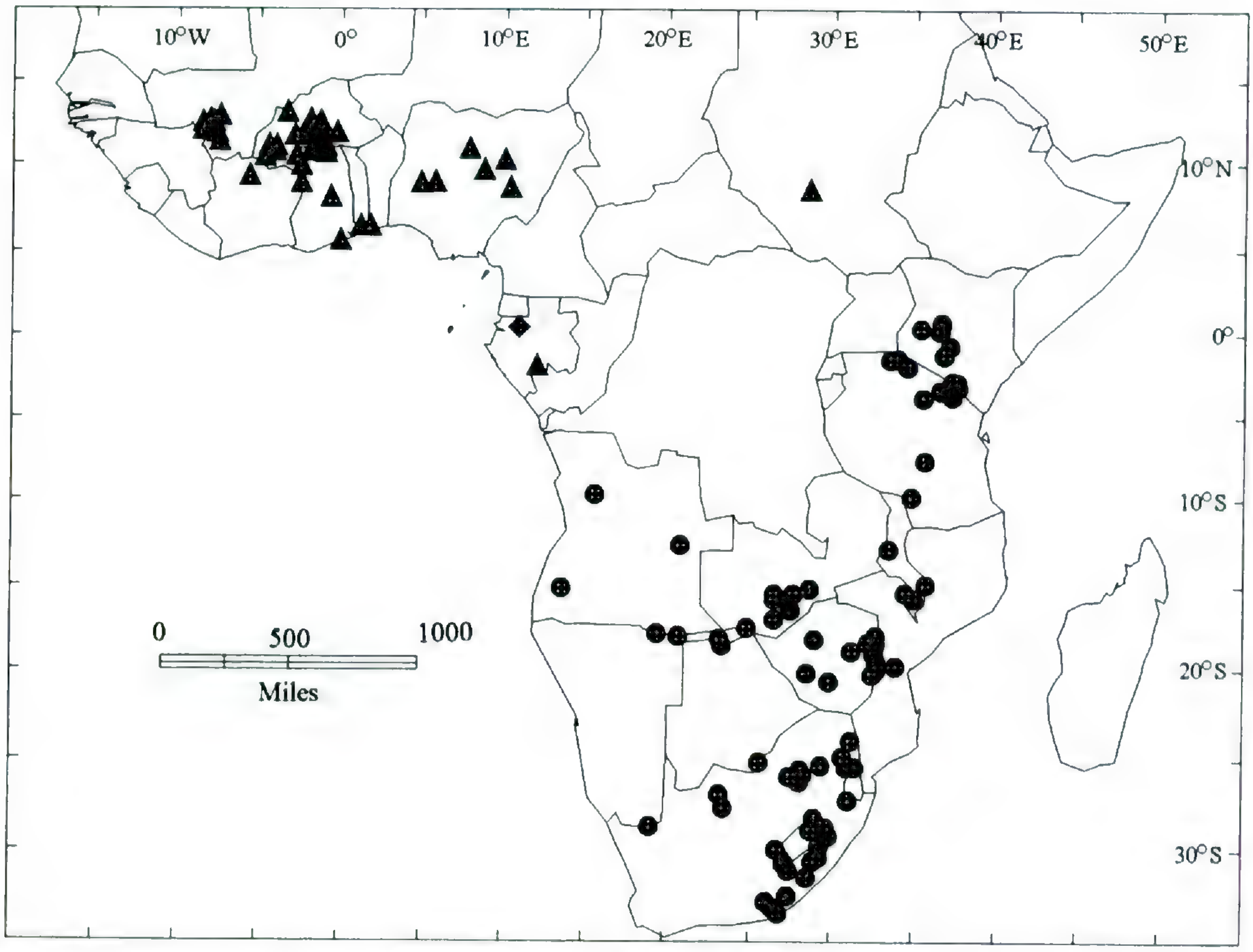


Figure 7. Distribution based on specimens of *Striga brachycalyx* (triangle), *S. elegans* (circle), and *S. hallaei* (diamond).

hogo, 10 km SW of Korhogo, 9°27′N, 5°38′W, *Riches 243* (K). MALI. **Koulikoro:** 12 km N of Oulessebougou, 12°0′N, 7°40′W, *Musselman & Mohamed* 9/30/88 (ODU). **Sikasso:** 6 km from Bougouni, 11°32′N, 7°30′W, *Raynal 22196* (K). NIGERIA. **Bauchi:** Bauchi Plateau, 10°25′N, 9°55′E, *Lely P567* (K). **Benue:** Abinsi, 7°20′N, 9°25′E, *Dalziel s.n.* (K). **Gongola:** Bogalo, close to River Benue, 8°45′N, 10°15′E, *Talbot 312* (K). **Kaduna:** Zaria, Samaru, 11°5′N, 7°43′E, *Harris 4, 7* (K). **Kwara:** Ilorin, 8°30′N, 4°32′E, *Parkinson* 9/17/86 (ODU). **Niger:** Bida/Mokwa Junction, 9°8′N, 5°40′E, *Mansfield* 9/4/78 (ODU). TOGO. **Southern:** Barani-Ideppe, 6°30′N, 1°45′E, *Kersting 665* (K). **Southern:** Togo Forest, 6°29′N, 1°9′E, *Anonymous s.n.* (US).

8. Striga chrysantha A. Raynal, Bull. Jard. Bot. Belg. 39: 378. 1969. TYPE: Central African Republic. Zemio-Rafai, *Descoings 12466* (holotype, P!).

Annual, small, < 12 cm tall, tufted, scabrous, densely pubescent, stem obtusely square. Leaves < 10 × 2(–3) mm, lanceolate, scale-like, shorter than internodes, margin entire, veins obscure. All bracts similar, 6 × 2 mm, lanceolate, shorter than calyx. Flowers alternate in rather dense spikes, spike as long as vegetative stem. Calyx 10-ribbed, 8–15 mm long; tube 6–13 mm long; lobes 5, subequal, deltate, 2 mm long, much shorter than tube. Corolla bright yellow, tube slender, 20–25 mm long, bent and expanded distally above calyx, glandular pubescent; lobes of lower lip 15 × 8 mm, spreading, broadly round, upper lip 8 × 10 mm, reflexed backward, emarginate.

Striga chrysantha is from the southern region of the Central African Republic and northern Democratic Republic of Congo (Raynal-Roques, 1969). It occurs between 3°40′N and 5°20′N (Fig. 8). The 10-calyx ribs, indumentum, and the yellow corolla suggest a relationship among this species, *S. lutea*, and *S. hirsuta*.

9. Striga dalzielii Hutch., Fl. W. Trop. Afr., ed. 1(2): 226. 1931. TYPE: Nigeria. Zungeru, *Dalziel 168* (holotype, K!).

Annual, 19–35 cm tall, stiffly erect, unbranched, scabrous, densely ciliate with long (1 mm), divergent hairs, stem square. Leaves 15–30 × 4–7 mm, 2-veined, opposite, linear, becoming reduced along the stem, as long as or longer than internodes, margin toothed. All bracts similar, 8–18 × 4 mm, linear, enclosing calyx, densely hispid along margins and midrib. Flowers sessile, imbricate, forming very dense spikes, spike shorter than vegetative stem. Calyx 10-ribbed, 5–8 mm long; tube 3–5 mm long; lobes 5, unequal, lanceolate, 2–3 mm long,

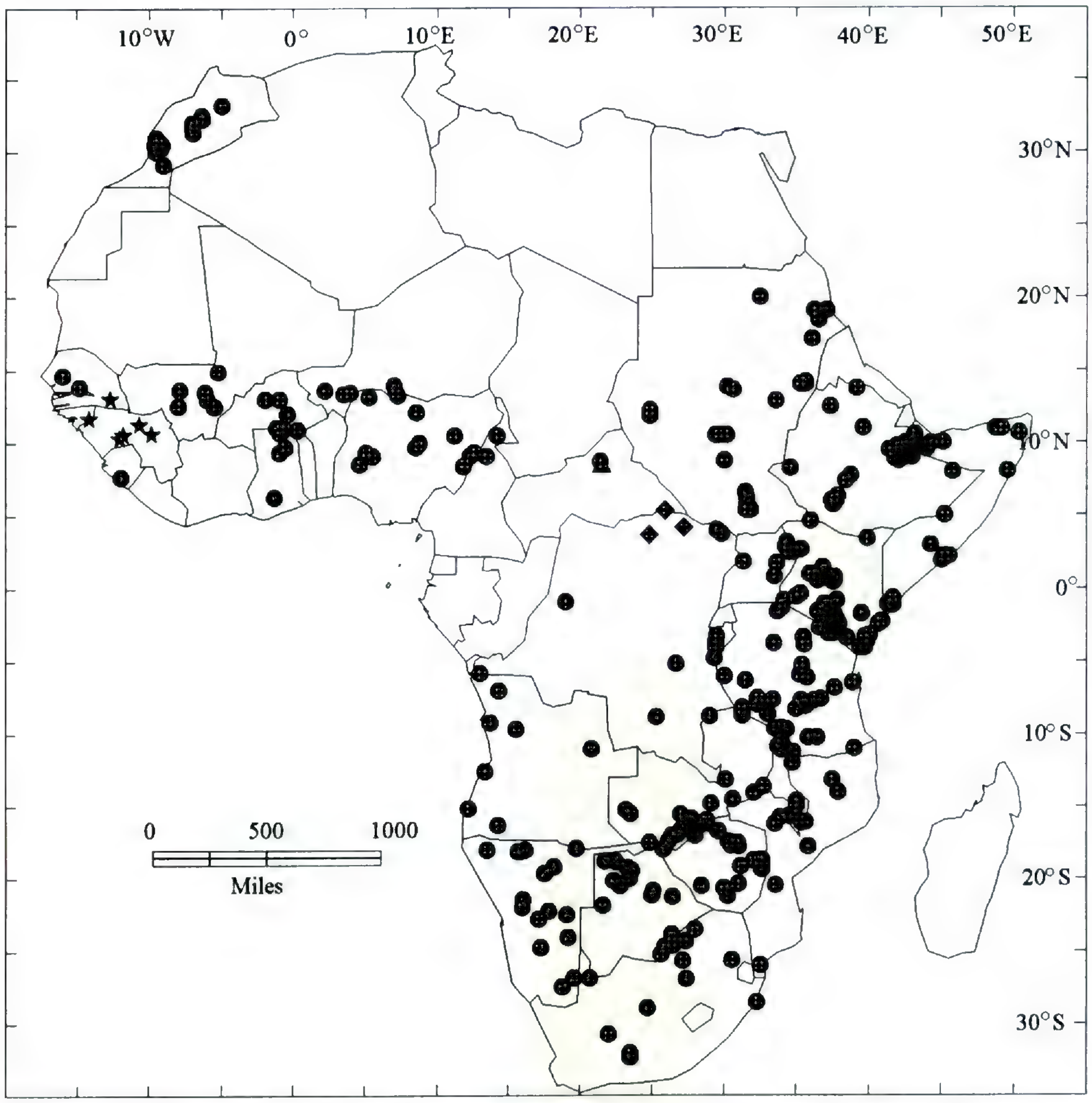


Figure 8. Distribution based on specimens of *Striga chrysantha* (diamond), *S. gastonii* (triangle), *S. gesnerioides* (circle), and *S. lepidagathidis* (star).

shorter than tube. Corolla pink, tube 10–20 mm long, bent and expanded distally above calyx, glandular-pubescent; lobes of lower lip 3–8 × 3–5 mm, obtuse, upper lip 3–6 × 6 mm, emarginate.

Striga dalzielii occupies the wet Guinea savannas in Mali, Guinea, and Nigeria (Raynal-Roques, 1987). It resembles *S. klingii* and *S. macrantha*, but *S. dalzielii* has 10-calyx ribs whereas *S. klingii* and *S. macrantha* have 10–15 ribs. *Striga dalzielii* is characterized by its leaf-like bracts and long hispid hairs.

10. Striga elegans Benth., Companion Bot. Mag. 1: 363. 1836. TYPE: South Africa. *Burke 443/3591* (holotype, K!).

Plant annual, 30(–50) cm tall, stiffly erect, unbranched or with 2 or 3 branches from base, scabrous, densely hispid, stem obtusely square, furrowed. Leaves 10–20(–30) × 2–3 mm, opposite, linear or lanceolate, shorter than internodes, margin entire, veins obscure. All bracts similar, 3–12 × 1–2 mm, lanceolate, shorter than calyx. Flowers opposite in dense racemes, raceme shorter than vegetative stem. Calyx 10–15-ribbed, 8–13 mm long; tube 6–7(–9) mm long; lobes 5 equal or 6 unequal, linear to lanceolate, 2–5 mm long, shorter than tube. Corolla brilliant scarlet, with yellow throat, tube 14–18 mm long, bent and expanded distally above calyx, densely glandular-pubescent; lobes of lower lip 5–10 × 3–5(–7) mm, ovate, upper lip 3–5 × 6–11 mm, emarginate.

Striga elegans is common in East and South Africa between 00°30′S and 33°30′S (Fig. 7). Its range extends from central Kenya south into Tan-

zania, Malawi, Zambia, Zimbabwe, Mozambique, Swaziland, Botswana, and Lesotho. *Striga elegans* shows considerable variation in corolla size and color that does not seem to correlate with geography. This beautiful species resembles *S. asiatica* and the two are sympatric in East Africa (see notes under *S. asiatica*), but it can be distinguished from *S. asiatica* by its relatively short, dense inflorescence with opposite flowers and large brilliant scarlet corolla lobes. Authentic crop loss from this parasite has not been reported.

Selected specimens examined. ANGOLA. **Huila:** Quilengues, Binde, 15°S, 13°30′E, *de Menezes A.377* (K). **Malange:** Pungo Andongo, 9°40′S, 15°30′E, *Welwitsch 5851* (K). **Moxico:** between River Namavumba and River Lukaia, 12°40′S, 20°45′E, *Milne-Redhead 4018* (K). BOTSWANA. **Ngamiland:** Leshums Valley, 18°30′S, 23°20′E, *Holub 1834* (K). **Southern:** Lobatse, 25°11′S, 25°40′E, *Tapscott 2548* (K). KENYA. **Central:** Nairobi, 1°20′S, 36°48′E, *Perceval 1901* (MO). **Coast:** Loitokitok, NE of Kilimanjaro 3°37′S, 37°20′E, *Rogers 560* (K). **Rift Valley:** Rumuruti, Laikipia, 50 km N of Colchecio Lodge, 0°19′N, 36°34′E, *Hepper & Jaeger 6618* (K, MO). LESOTHO. **Northern:** Mont-aux-Sources, 28°56′S, 28°50′E, *Grant 2212387* (MO). MALAWI. **Central:** Kasungu, Chipala Hill, 4 mi. N of Kasungu, 12°55′S, 33°29′E, *Robson & Jackson 1179* (K). MOZAMBIQUE. Paraia Sepulveda, *Lavranos 4265* (MO). NAMIBIA. **Kavango:** 2.4 mi. E masari Camp on road to Nyangana, 18°0′S, 20°41′E, *de Winter 4087* (K). SOUTH AFRICA. **Bechuanaland:** Little Klibbolikhonni, 27°0′S, 23°15′E, *Burchell 2482* (K). **Cape:** Albany, Woest Hill, 33°0′S, 26°20′E, *Bayliss 4526* (GH, MO). **Karasburg:** Orange River Colony, 28°45′S, 19°0′E, *Walich 1278* (K). **Natal:** between Mooi River and Estcourt, 29°18′S, 29°56′E, *Wood 394* (K). **Orange Free State:** Walton, 12 km S of Harrismith, 28°18′S, 29°3′E, *Jocobsz 218* (K). **Transkei:** Griqualand, 30°40′S, 29°0′E, *Tyson 1368* (MO). **Transvaal:** Randburg, Kelland, 26°6′S, 27°59′E, *Liebenberg 8581* (MO). SWAZILAND. **Louwsburg:** 2 mi. E of Goedgegun, 27°20′S, 31°7′E, *Ross 1743* (K, MO). TANZANIA. **Arusha:** Arusha National Park, 3°25′S, 36°37′E, *Richards 25019* (K). **Iringa:** 8 km E of Kilolo, which is 30 km SE of Iringa, 7°40′S, 35°40′E, *Peterson 833* (K). **Kilimanjaro:** E Kilimanjaro Mountain, 30°0′S, 37°38′E, *Haarer 637* (K). TANZANIA. **Mara:** Chamliho Hill, Ikizu Musoma, Lake Province, 1°30′S, 33°58′E, *Tanner 1690* (K). **Iringa:** Kyimbila, N of Lake Nyasa, 9°50′S, 34°50′E, *Stolz 2494* (GH, MO). ZAMBIA. **Lusaka:** 129 km E of Lusaka, 15°15′S, 28°40′E, *Robinson 6174* (K). **Southern:** Kalomo, Kanchele, 17°0′S, 26°30′E, *Astle 1571* (K). ZIMBABWE.**Maincaland:** 6 mi. N of Troutbeck, Inyanga, 18°21′S, 32°15′E, *Robinson 1961* (K). ZIMBABWE. **Mashonaland South:** Harare, Circular Drive, 17°50′S, 31°3′E, *Hughes s.n.* (ODU). **Matabeleland South:** Belingwe (Mberengwa), Mount Buhwa, 20°37′S, 29°55′E, *Wild 4315* (K). **Midlands:** Gokwe, 18°9′S, 29°0′E, *Bingham 1178* (MO).

11. Striga forbesii Benth., Companion Bot. Mag. 1: 364. 1836. TYPE: Mozambique. *Forbes s.n.* (holotype, K!).

Annual, 37–72 cm tall, stiffly erect, unbranched or with 2 to 4 branches below middle, scabrous to hispid, stem square, furrowed. Leaves 15–40(–90) × 4–12 mm, opposite, lanceolate, coarsely toothed, 3- to 5-veined, usually shorter than internodes. Lower bracts 20–50(–90) × 3–12 mm, leaf-like, longer than calyx, upper bracts shorter than calyx. Flowers opposite in open racemes, raceme shorter than vegetative stem. Calyx 10–15-ribbed, 9–14 (–20) mm long; tube 4–6 mm long; lobes 5, equal, broadly lanceolate, 5–9(–14) mm long, longer than tube. Corolla salmon-pink, tube 20–25 mm long, bent and expanded distally above calyx, densely glandular-pubescent; lobes of lower lip 6–9 × 3–6 mm, obovate, spreading, upper lip 3–6 × 6–9 mm, emarginate.

Striga forbesii ranges between 14°30′N and 28°30′S (Fig. 9). It is common in the Sudanian savannas from Senegal to Somalia and extends into East Africa reaching its southern limits in the Republic of South Africa. A plant of relatively wet open areas and swampy soils, *S. forbesii* can cause damage to irrigated crops such as sugar cane in Somalia (Musselman, unpublished).

Knepper et al. (1991) showed that some populations of *S. forbesii* are distylous. Flowers either had styles strongly exserted from the corolla tube exposing the stigma, or stigmas were among or just below the epipetalous stamens, with increasing possibilities of self-pollination.

Striga forbesii resembles *S. latericea.* Both are sympatric in East Africa, occupy wet habitats, and have unique salmon-pink corollas. No work has been done to demonstrate interfertility between these two species. *Striga forbesii* is distinguished from *S. latericea* by its annual habit, stiff hairs that give the plant a harsh texture, coarsely toothed and broader leaves, and shorter corollas with smaller lobes and a brighter salmon-pink color. The corolla color is darker in *S. latericea.*

Selected specimens examined. ANGOLA. **Cuanza North:** Cuanza, 8°50′S, 15°0′E, *Johnston 83* (K). **Moxico:** between River Masanu and River Katetele, 13°0′S, 21°0′E, *Milne-Redhead 4155* (BM, K). BOTSWANA. **Chobe:** near Pandamentenga, 18°9′S, 24°24′E, *Musselman 4/9/86* (ODU). **Ngamiland:** NE Maun, 20°0′S, 23°26′E, *Lambrecht 479* (K). **Bujumbura:** Karazi, 3°23′S, 29°22′E, *Lewalle 335* (K). **Ruand:** Kigungee Territory, Biumba, 2°12′S, 30°32′E, *Alcool 4142* (K). CAMEROON. **Northern:** 5 km W Marona, 10°35′N, 14°0′E, *de Wilde et al. 2954* (K). DEMOCRATIC REPUBLIC OF CONGO. **Bandundu:** Kisambo, Kwango, 6°25′S, 18°10′E, *Anonymous 3384* (K). **Equateur:** Olru-Uele, 4°0′N, 22°35′E, *Lebrun 3557* (GH). **Haut Zaire:** Oriental, Dungu, Garamba National Park, 3°40′N, 28°30′E, *Jaeger 1225* (K). **Katanga:** Elisabethville, 11°40′S, 27°28′E, *Quarre 1451* (K). **Oriental:** Kasai, Gandajika, 6°42′S,

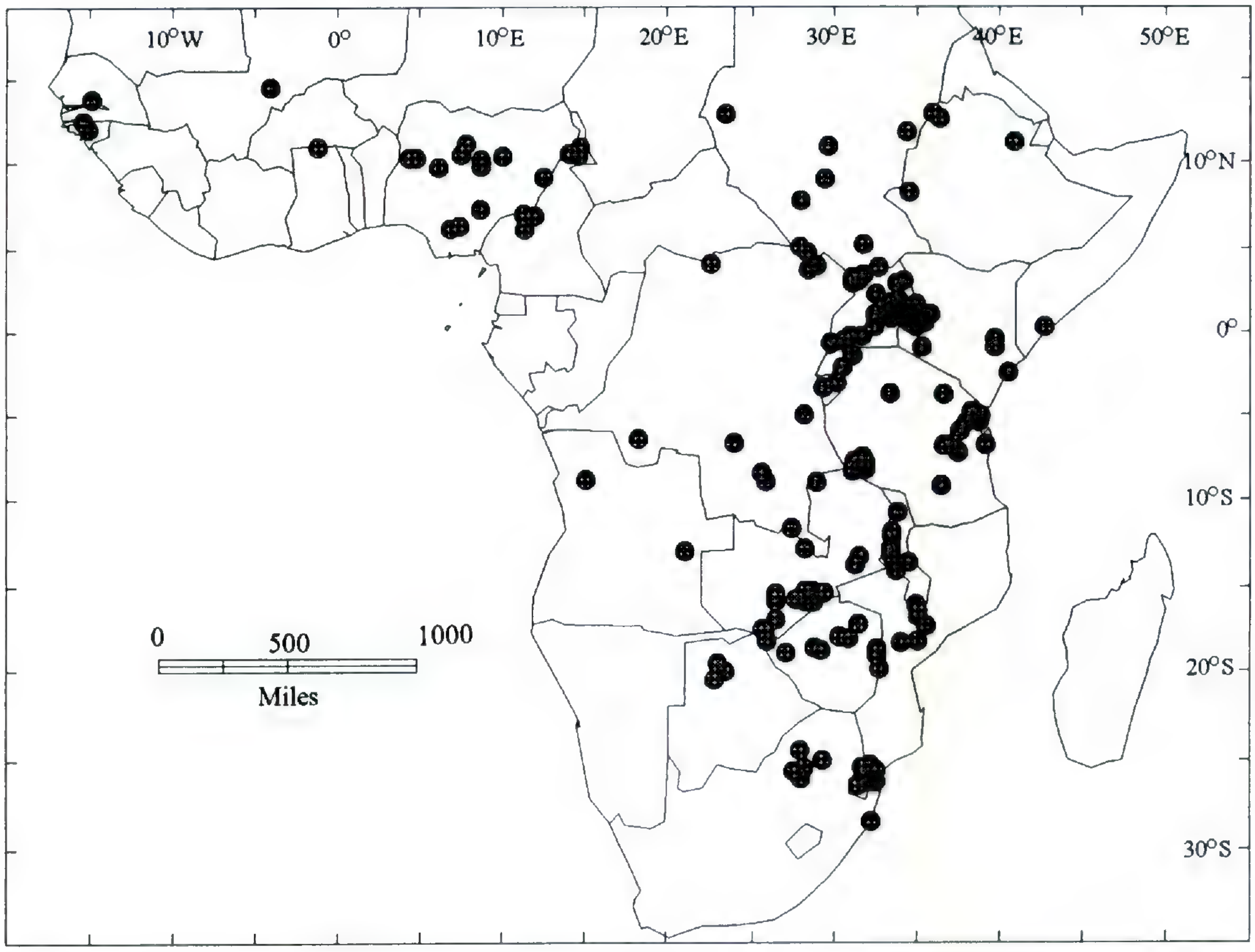


Figure 9. Distribution based on specimens of *Striga forbesii* (circle).

34°0′E, *Liben 2038* (K). **Shaba:** River Lualaba, 50 km downstream from Bukama, 8°55′S, 25°55′E, *Paterson 59* (K). ETHIOPIA. **Ilubabor:** Ilubabor, Gambela, Baro River, 8°16′N, 34°35′E, *Ash 544* (K). **Welo:** Asaeta, Awash Valley, 33 km S of Tandah, 11°17′N, 40°54′E, *Ash 2259* (K). GAMBIA. **Eastern:** Kuntaur, 13°49′N, 14°52′W, *Ruxton 67, 149* (K). **Upper:** Bolgatanga, Crocodile Pool, 10°50′N, 0°50′W, *Hall 558* (K). GUINEA BISSAU. **Northern:** between Farim and Bigene, 12°10′N, 15°15′W, *Anonymous 3063* (K). **Western:** Sao, Fa, 12°27′N, 16°17′W, *Guerra 3812* (K). KENYA. 30 mi. W of Bwa, *Wham 26/2/24* (K). **Coast:** Kipini, Mlango Ya Simba, 2°30′S, 40°32′E, *Greenway & Rawlin 9463* (K). **Narok:** Mara Plains, 1°0′S, 35°20′E, *Stewart 327* (K). **Rift Valley:** Chyulu-Goohills, 1°0′N, 35°50′E, *Bally 8072* (K). **Western:** Uasingishu Plateau and Trans Nzoia, 0°30′N, 34°35′E, *Dowson 674* (K). MALAWI. **Central:** 2 mi. N of Mzambazi, 13 mi. S of Mperembe, 11°58′S, 33°33′S, *Pawek 13954* (K). **Kyimbila:** Nyasa Hochland-Station, *Stolz 213* (GH, US). **Northern:** 16 mi. N of Rumpi, 10°45′S, 33°54′E, *Pawek 13913* (K). **Southern:** Lower Shire Valley, Elephant Marsh, 16°20′S, 35°0′E, *Robertson 5* (K). MALI. **Massina:** Mopti, 14°30′N, 4°8′W, *Lean 37* (K). MOZAMBIQUE. **Maputo:** Maputo (Lourenco Moazgues), Vila Luisa, 25°45′S, 32°35′E, *Grandraux & Delemos 7913* (K). **Sofala:** near Massara, 18°20′S, 34°5′E, *Anonymous 1858* (K). **Zambezia:** Morrumbala Plains, 17°22′S, 35°36′E, *Kirk* 15/1/63 (K). NIGERIA. **Amambra:** Udi, upper Nau River, 6°18′N, 7°20′E, *Anderson 1364* (K). **Bauchi:** Bauchi Plateau, 10°27′N, 9°57′E, *Lely P369* (K). **Bendel:** 3 mi. N Asaba, 6°7′N, 6°47′E, *Killick 234* (K). **Cross River:** Oban, 5°17′N, 8°35′E, *Talbot 308* (BM). **Gongola:** Mambilla Plateau, 6°5′N, 11°14′E, *Chapman 2935* (K). **Kaduna:** 2 mi. NE of Kaduna, 10°32′N, 7°26′E, *Jackson 254* (K). **Niger:** Zungeru, 9°48′N, 6°5′E, *Dalziel 167* (K). **Plateau:** Jos Plateau, 9°50′N, 8°40′E, *Batten-Poole 371* (K). **Sokoto:** 10 mi. N Bussa, 10°20′N, 4°35′E, *Cook 386* (K). SOMALIA. **Jubba House:** Middle Juba, Mareeri, 0°25′N, 42°42′E, *Musselman 7086* (ODU). SOUTH AFRICA. **Natal:** Inanda, 26°7′S, 28°3′E, *Wood 440* (K). **Qriqualand:** N of Kaap, Barberton, 28°30′S, 23°45′E, *Thorneiop 2206* (K). **Transvaal:** Crocodile River, beyond Brits, 25°42′S, 27°45′E, *Dyer & Merdoom 3421* (K). SUDAN. **Bahr Al-Ghazal:** Wau, 7°46′N, 28°2′E, *McCintosh 57k, 109k* (K). **Equatoria:** Mount Nakbi between Yambio and Tambura, 5°0′N, 28°5′E, *Myers 6997* (K). **Jungali:** 20 km N of Kongor, 7°10′N, 31°21′E, *Lock 82/31* (K). **Kassala:** Metemma, Gallabat, 12°59′N, 36°0′E, *Schweinfurth 179* (K). **North Darfur:** Darfur, Zalingei, 12°45′N, 23°29′E, *Lynes 577* (BM). **South Kurdufan:** Kadugli, Nuba Mountains, 11°1′N, 29°41′E, *Bashir s.n.* (K). SWAZILAND. **Central:** Tshaneni, Swaziland Irrigation Scheme, 26°30′S, 31°30′E, *Parker 1066* (ODU). TANZANIA. **Morogoro:** Kilosa, 6°50′S, 36°50′E, *Robertson 676* (K). **Pwani:** Dar es Salaam, University College, 6°47′S, 39°18′E, *Mwasumbi 10487* (K). **Rukwa:** Milepa, 8°3′S, 31°56′E, *Lea LR20* (K). **Shinyanga:** Shinyanga, 3°45′S, 33°27′E, *Koritschoner 2059* (K). **Tanga:** Handeni, 5°27′S, 28°2′E, *Ryae H40/33* (K). **Ziwa:** Bukoba, Kakindu, 1°30′S, 31°10′E, *Haarer 2307* (K). **Zanzibar:** Pemba, *Vaughan 402, 1613, 1664* (K). UGANDA. **Busoga:** Bugerere, 0°45′N, 33°30′E, *Liebenberg 1532* (K). **Eastern:** Elgon, Pemba, 1°10′N, 34°30′E, *Fames 1905* (K). **Karamoja:** Honyili Ridge, 2°25′N, 33°40′E, *Wilson 880* (K). **Nile:** Arua West Hill, 3°1′N, 31°10′E, *Hazel 442* (K). **North Buganda:** Kakoge, Mengo, 1°0′N, 32°30′E, *Brown 1732* (K). **Northern:** Lango, 2 km W of Ngunyboke, 2°13′N, 32°34′E, *Lye 4747*

(K). **South Buganda:** Masaka Rest Camp, 0°20′S, 31°44′E, *Chancellor 72* (K). **Southern:** Mbarara, 0°37′S, 30°39′E, *Maitland 103* (K). ZAMBIA. **Copperbelt:** Kitwe, 12°54′S, 28°17′E, *Fanshawe 10003* (K). **Eastern:** Luangwa Valley, 13°20′S, 31°35′E, *Astle 4471, 4570* (K). **Luapula:** 5 km E of Lusaka, 15°28′S, 28°19′E, *King 260* (K). **Shaba:** 3 mi. NE Ndola-Waterlogged Dambo, 12°58′S, 28°46′E, *Draper 25* (K). **Southern:** Kafue River, at Lubalansuki, Namwala District, 15°50′S, 26°30′E, *Mitchell 24/78* (K). ZIMBABWE. **Manicaland:** Melsetter, 6 mi. S Mutambara Village, 19°50′S, 32°47′E, *Whiteside 107793* (K). **Mashonaland North:** Mazore, Shamva, near Lions Heads, 17°17′S, 31°30′E, *Wild 599* (K). **Mashonaland South:** Makwiro, 18°0′S, 30°20′E, *Smith 22673* (K). **Matabeleland North:** Urungwe National Park, 271 km from Salisbury on Chirundu Road, 18°57′S, 27°6′E, *Philcox et al. 8796* (K). **Midlands:** Kwe Kwe, 18°55′S, 29°49′E, *Musselman 124* (K).

12. Striga gastonii A. Raynal, Bull. Mus. Natl. Hist. Nat., B, Adansonia, 3: 225–227. 1987. TYPE: Chad. Danamadji, *Audru 1245* (holotype, P not seen; isotype, ALF not seen).

Perennial, 7 cm tall, succulent, tufted, glabrous, stem obtusely square. Leaves scale-like, 1–4 × 1–2 mm, lanceolate, appressed, longer than internodes, margin entire, veins obscure. All bracts similar, 3 × 1–2 mm, lanceolate, shorter than calyx. Spike dense, longer than vegetative stem. Calyx 5-ribbed, 3–5.5 mm long; tube 2–4 mm long; lobes 5, subequal, subulate, 1–2 mm long, shorter than tube. Corolla purple, tube 8 mm long, bent and expanded distally above calyx, sparsely glandular-pubescent, weakly bilabiate; lobes of lower lip 4 × 1–2 mm, obovate, upper lip 1 × 1–2 mm, emarginate, tips obtuse.

Striga gastonii is endemic to the lateritic plains in southern Chad and Central African Republic (Fig. 8) and is parasitic on *Lepidagathis* (Acanthaceae) species (Raynal-Roques, 1987). It is a small plant that resembles *S. gesnerioides* var. *minor* Santojuan in its overall appearance. The relationship of *S. gastonii* to the *S. gesnerioides* parasitizing *Lepidagathis* in India (e.g., Saldanha, 1963) should be investigated. *Striga gastonii* may be a host-specific strain of *S. gesnerioides.*

13. Striga gesnerioides (Willd.) Vatke, Oesterr. Bot. Z. 25: 11. 1875. *Buchnera gesnerioides* Willd., Sp. Pl. 338. 1801. TYPE: India. *Koenig s.n.* (B 11573 not seen).

Buchnera orobanchoides R. Br., Flora 2: 388, t.2. 1832. *Striga orobanchoides* (R. Br.) Benth., Companion Bot. Mag. 1: 361. 1836. TYPE: Ethiopia. *Salt s.n.* (holotype, BM!).

Striga orchidea Hochst., Fl. XXIV. 43. 1841. TYPE: *Kotschy 387* (holotype, K!).

Striga gesnerioides var. *minor* Santapau, Kew Bull. 1948: 491. 1949. TYPE: *Santapau 3072* (holotype, Blatter Herb., St. Xavier's College, Bombay not seen).

Annual or occasionally perennial, 12–30 cm tall, unbranched or branched from the base, usually succulent, drying black or brown, sparsely to densely hispid or pilose, stem terete or obtusely square. Leaves 4–10 × 1–3 mm, lanceolate, opposite/subopposite, scale-like, appressed, usually shorter than internodes, margin entire, veins obscure. All bracts similar, 2.5–6 × 2–3 mm, lanceolate, longer or shorter than calyx. Flowers opposite/subopposite in simple spikes, spike shorter or longer than vegetative stem. Calyx 5-ribbed, 4–9 mm long; tube 3–6 mm long; lobes 5, unequal or subequal, linear, deltate or lanceolate, 1–3 mm long, shorter than tube. Corolla creamy-white, blue, pink, purple, tube 8–14 mm long, bent and expanded distally above calyx, pubescent, with very few hairs or glandular hairs; lobes of lower lip 2–6 × 1.3–3 mm, obovate, spreading, upper lip 1–2 × 2–2–5 mm, indistinctly bilobed or emarginate, usually wider than long.

Striga gesnerioides is the most widely distributed of all witchweed species (Fig. 8) and is found between 33°10′N and 32°15′S, extending into Arabia and Asia. *Striga gesnerioides* was introduced into Florida in the United States (Sand et al., 1990) but has not become an agronomic problem.

Striga gesnerioides parasitizes only dicotyledons. Host selection, succulence, reduction of leaves, and tufted habit have been considered (see Boeshore, 1920) to represent a reduction in the direction of the holoparasitic Orobanchaceae. Thalouarn et al. (1991) found that *S. gesnerioides* has a weak capacity to fix CO_2. These features of morphology, host selection, and physiology distinguish *S. gesnerioides* from other members of the genus.

Selected specimens examined. (All collections of *S. gesnerioides* are listed here. Under each strain we have listed specimens representative of those strains). ANGOLA. **Benguella:** Benguella, 12°36′S, 13°20′E, *Gossweller 3540* (K). **Cunene:** Cahama, 16°19′S, 14°15′E, *Pearson 2421* (K). **Luanda North:** Catete, 9°2′S, 13°42′E, *Anonymous 9200* (K). **Lunda South:** Cazange, 11°2′S, 20°45′E, *Gossweller 647* (K). BOTSWANA. **Central:** Boteti Delta Area, S of Mopipi, 20°50′S, 25°5′E, *MacDonald 21* (K). **Desert:** 5 km NW of Bokspits, 26°51′S, 20°41′E, *Skarpe 52* (K). **Ghanzi:** 13 mi. S of Ghanzi on Lobatsi Road, 21°50′S, 21°36′E, *Brown 8282* (K). **Kweneng:** Artesia, 24°2′S, 23°26′E, *Mitchison* 1975 (K). **Ngamiland:** Mboma Island, 19°12′S, 23°18′E, *Smith 830* (K). **Southern:** Gaborone, Aedume, 24°42′S, 25°54′E, *Hansen 3375* (K). BURKINA FASO. **Central:** Korsimoro, 30 km from Kaya, 12°0′N, 1°0′W, *Raynal 22603* (K). **Northern:** 55 km from Ouahigouya, 13°0′N, 2°0′W, *Raynal 22613* (K). BURUNDI. **Bujumbura:** Magari, 4°44′S, 29°18′E, *Reekmans 9834* (K). **Rubindi:** Bururi, 3°56′S, 29°33′E, *Reek-*

mans 6926 (K). **Rusizi:** 14 km from Bujumbura, 3°20′S, 29°27′E, *Lewalle 1474* (K). CAMEROON. **Northern:** 5 km W of Maroua, 10°37′N, 14°12′E, *de Wilde & de Wilde-Duyfjes 29629* (K). CENTRAL AFRICAN REPUBLIC. **Bamingui-Bangoran:** Saline on Niao River, 2 km from confluence with Ground River, 8°42′N, 21°22′E, *Fay 3354* (K). CONGO. **Shaba:** Lubanda, Kivu Province, 5°10′S, 26°38′E, *Germain 5717* (K). DEMOCRATIC REPUBLIC OF CONGO. **Bandundu:** Tweya, 0°56′S, 18°58′E, *Robyn 1980* (K). **Haut Zaire:** Faradje-Olru, 3°44′N, 29°43′E, *Lebrun 3535* (MO). **Shaba:** Luvngu, 8°48′S, 25°17′E, *Linder 1966* (K). ETHIOPIA. **Arsi:** Lake Langano, 7°45′N, 38°45′E, *Ash 347* (K). **Gamo Gofa:** 12 km S of Arba Minch, 6°5′N, 37°40′E, *Gilbert et al. 345* (K). **Gonder:** Tana, 12°30′N, 37°20′E, *Pichi-Sermolli 1296* (K). **Ilubabor:** Gambela, 8°18′N, 34°37′E, *Ash 557, 1089* (K). **Ogaden Harerge:** E of Grawa, 8°50′N, 42°25′E, *Burger 2846* (K, US). **Shewa:** SW Shore of Lake Langano, 7°25′N, 38°25′E, *Gilbert & S.B. 1078* (K). **Tigray:** Takaze Valley, 13°43′N, 39°11′E, *Gilbert & Getachew 2958* (K). GAMBIA. **Eastern:** Kuntaur, 13°45′N, 14°40′W, *Ruxton 53* (K). GHANA. **Northern:** Tamale, 9°20′N, 0°59′W, *Irvine 4572, 4574* (K), *Hall 2058* (K). **Upper:** Bolgatanga, 10°45′N, 0°55′W, *Parker 2023* (K). **Volta:** Keta, Rest House, 5°55′N, 1°2′E, *Akpabla 36* (K). KENYA. **Coast:** Arabuko, Kilifi, 3°40′S, 39°50′E, *Graham 1675* (K). **Eastern:** Buna, 3°26′N, 39°54′E, *Gilbert 13104* (K). **Nyanza:** Karungu, Homa Mount Foot, 0°49′S, 34°9′E, *Twuner 6730* (K). **Rift Valley:** Chebloch, Eudo, Upper Kerio Valley, 0°22′S, 35°15′E, *Tweedie 2448* (K). MALAWI. **Northern:** 19 mi. S of Chilumba, 11°0′S, 33°54′E, *Pawek 9601* (K). **Southern:** Chiwembu, Mankhudwe Hill, 15°38′S, 34°32′E, *Smith et al. 5872* (K); Sombani Plateau, Mulanje Mountain, 16°10′S, 35°33′E, *Mouculie 574* (K). MALI. **Koulikoro:** Sotuba, 12°50′N, 7°59′W, *Raynal 22063* (K). **Massina:** Dioura, N Macina, 14°52′N, 5°16′W, *Davey s.n.* (K). **Segou:** between Dougouba and Markala, 13°0′N, 6°0′W, *Raynal 22565* (K). **Sikasso:** 4 mi. from Koutiala, 12°30′N, 5°30′W, *Raynal 22211* (K). MOROCCO. **Agadir:** Agadir, 30°30′N, 9°40′W, *Hettewy 52, 179, 186* (K), *Lynes 53* (BM). **Azilal:** near Demnate, 31°40′N, 7°3′W, *Balls 3022* (K). **Beni-Mellal:** Kesbeh, 32°20′N, 6°20′W, *Lewalle 10634* (BM). **Goulimime:** 1 km E of Tirhmi, 29°6′N, 9°5′W, *Miller et al. 615* (BM). **Quaovizert:** Moyen Atlas Mountain, 33°10′N, 5°0′W, *Jahandiez 149* (BM). **Southwest:** Oued Massa, between Agadir and Tiznit, 3°N, 9°35′W, *Davis 53515* (BM). MOZAMBIQUE. Liben, *Kuntze 94* (K). **Maputo:** Maputo (Lourenco Marques), 25°58′S, 32°32′E, *Balsinhas 152* (K). **Niassa:** Marrupa, slope of Mountain Kuwanko, 15 km on road to Nungo, 13°8′S, 37°30′E, *Jansen 8040* (K). **Sofala:** Chibabava, Lown Buzi, 20°25′S, 33°35′E, *Swynnerton 1413* (K). **Tete:** Tete, 16°13′S, 33°33′E, *Kirk* (K), *Peters 8* (K). NAMIBIA. Along road to Ethosha Pans National Park, *Musselman & Visser* 4/2/86 (ODU). **Gobabis:** 10 km E of Gobabis, 22°28′S, 19°5′E, *Wanntop 760* (K). **Great Namaqualand:** Jafelberg, 24°45′S, 17°15′E, *Smith 26* (K). **Grootfontein:** 11 mi. from Otavi-Grootfontein on flat between mountains, 19°38′S, 17°30′E, *Tolken & Hardy 919* (K). **Kaokoland:** Mopane, Otjivero, 18°0′S, 13°29′E, *Winter & Leistner 5368* (K). **Karasburg:** Grot Karasberg, Naruda Sud Ravine, 27°29′S, 18°45′E, *Pearson 8122* (K). **Karibib:** Damaraland Otisondu, 22°0′S, 15°56′E, *Seydel 4491* (K, US). **Kavango:** bank of Okavango River at Runyu, 17°56′S, 19°44′E, *Winter & Marais 4479* (K). **Keetmanshoop:** Keetmanshoop, 3 mi. W of Aroab, 26°51′S, 19°35′E, *Winter 3372* (K). **Mariental:** E Aranos, Farm Mara on the Botswana border, 24°2′S, 19°10′E, *Vuuren & Giess 1132* (K). **Omaruru:** Farm Okandjon North, 30 km NW of Omaruru, 21°26′S, 16°0′E, *Wanntop 814* (K). **Windhoek:** 6 mi. E of Homestead on Nossob River, Gibeon, Farm Haruchas, 22°15′S, 17°48′E, *Leistner 1817* (K). **Windhoek:** Avis Dam, 22°46′S, 17°7′E, *Liebenberg A516* (K). NIGER. **Maradi:** 10 km NW of Maradi, 13°45′N, 7°0′E, *Parker 2044* (K). **Niamey:** Niamey, 13°35′N, 2°10′E, *Olufsen 479* (K). NIGERIA. **Bauchi:** 10 mi. from Gombe on Biu Road, 10°27′N, 11°14′E, *Daramola 62346* (K). **Gongola:** Kilba Hills and Yola, 9°20′N, 12°35′E, *Dalziel 300* (K). **Kwara:** Ilorin, 36 mi. from Ilorin, on Ilorin–Bacita Road, 8°30′N, 4°35′E, *Gbile et al. 63394* (K). **Niger:** between Mokwa and Bida, 9°3′N, 5°30′E, *Lowe A12* (K). **Oyo:** Ado Rock, 7°40′N, 5°15′E, *Keay 25446* (K). **Plateau:** Jos Plateau, 9°40′N, 8°35′E, *Lely 535* (K). RUANDA. **Southern:** Rumonge, along Lake Tanganyika, 3°58′S, 29°25′E, *Shantz & Tanner 4196* (K). SENEGAL. Djembering, *Broadbent 158* (K), *Farmer 131* (K). SIERRA LEONE. **Western:** Buyabuya, 12°10′N, 7°50′W, *Elliott 4279* (K). SOMALIA. **Bari:** Galgallo, 64 km SW of Bosaso, 10°58′N, 49°1′E, *Bally & Melville 15841* (K). **Bay:** Buur Heybo (bur Eibi), 2°59′N, 44°16′E, *Thulin & Mahamed 7088* (K). **Jubbada Hoose:** 16 km S of Badade, 1°4′S, 41°23′E, *Gillett et al. 25136* (K). **Mogadisho:** Mogadishu International American School, 2°7′N, 45°32′E, *Kuchar 15312* (K). **Nugaal:** Nugaal Valley, 8°5′N, 49°35′E, *Hemming & Watson 30216* (K). **Sanaag:** Sugli, 10°58′N, 48°53′E, *Ballenetto 277* (K). **Woqooyi Gelbeed:** Borama, 10°2′N, 43°10′E, *Gilbert 4723* (K). SOUTH AFRICA. **Cape:** bank of Kareiga River, Bathurst District, 32°15′S, 23°30′E, *Bayliss 5212* (GH). **Natal:** Inomda, Amonopl Grass, *Wood 4276* (K). **Orange Free State:** Kimberly, Dronfield, 28°54′S, 24°41′E, *William 5975* (K). **Transvaal:** 9 mi. W of Krugersdorp on Farm Gladysvale, 26°5′S, 27°24′E, *Robin 3839* (K). SUDAN. **Bahr Al-Gebel:** Terakekka, 5°25′N, 31°42′E, *N.D.H 7361* (K). **Equatoria:** 10 km S of Bemmeiza, 40 km S of Bor, 5°55′N, 31°36′E, *Lock 82/44* (K). **Kassala:** Gedaref, Um Shagara, 14°2′N, 35°30′E, *Beshir 151* (K). **Lake Province:** near Baidit, 35 km N of Bor by Kongor Road, 6°30′N, 31°30′E, *Lock 81/77* (K). **North Kurdufan:** Barah, Hadeid Sherifi, 13°38′N, 30°36′E, *Wickens 536* (K). **Red Sea:** Erkowit, 18°27′N, 36°32′E, *Jackson 2722* (K). **South Darfur:** 13 km S Nyala, 11°58′N, 24°51′E, *Wickens 3159* (K). **South Kurdufan:** G.T.Z. Farm, near Telo, 10°30′N, 30°E, *Musselman 6174* (K, ODU). **Upper Nile:** Bahr el Jebel, Sheikh Jowbe, 8°45′N, 30°E, *Brown 1644* (K), *Shellai 837* (K). TANZANIA. 1.5 mi. NE Dangani, *Drummond & Hemsley 3319* (K). **Arusha:** Lake Tanganyika, Mbulu, 3°52′S, 35°33′E, *Richards 4512* (K). **Dodoma:** Handeni, Kwa Mtono, 5°15′S, 35°25′E, *Archbold 2776* (K). **Iringa:** 19 km E of Ibuguziwa Ferry, 7°55′S, 35°35′E, *Bjronstad 1363* (K). **Kigoma:** Mahali Mountains, 6°0′S, 30°E, *Jefford et al. 53* (K). **Kilimanjaro:** Mrau Rombo, E Kilimanjaro, 3°7′S, 37°30′E, *Haarer 5127* (K). **Mara:** Lake Province, Musoma Town, 1°30′S, 33°48′E, *Tanner 3996* (K). **Mbeya:** Galula, S Saza, 8°37′S, 33°2′E, *Carter et al. 2479* (K). **Morogoro:** 10 km from base of Kitonga Gorge, 7°40′S, 36°15′E, *Lovett & Congdon 1404* (K). **Mpana:** Katabi Plains, 6°20′S, 31°30′E, *Sanane 1571* (K). **Mtwara:** Tunduru, 60 mi. from Masasi, 11°0′S, 39°0′E, *Richards 17965* (K). **Rukwa:** Rukwa Valley, 7°40′S, 32°20′E, *Pielou 130* (K). **Ruvuma:** 6.5 km E of Gumbiro, 10°15′S, 35°52′E, *Milne-Redhead & Taylor 10018* (K). **Shinyanga:** Shinyanga,

3°45′S, 33°27′E, *Burtt 3770* (K). **Singinda:** Doma, 7 mi. on Bahi to Kilimatinde Road, 5°54′S, 35°14′E, *Bidgood et al. 1204* (K). **Ufipa:** Kalambo Falls on Tanzania side of the river, 8°37′S, 31°15′E, *Richards 12813* (K). TOGO. **Northern:** near Dapaon (Dapango), 10°53′N, 0°16′E, *Ern et al. 1478* (K). UGANDA. **Busoga:** 2 mi. N of Nkondo–Kigindi Road, 0°50′N, 33°30′E, *Wood 805* (K). **Eastern:** Soroti Rock, 1°43′N, 33°38′E, *Lye 5379* (K). **Karamoja:** Lodoketeminit, near Moroto, 2°30′N, 34°36′E, *Kerfoot 1313* (K). **Northern:** N of Mengo, 2°N, 32°28′E, *Brown* 2185 (K). **Western:** Butiaba, Lake Albert, 1°50′N, 31°20′E, *Thomas 3458* (K). ZAMBIA. **Central:** Lunzua Valley, Kafakula, 15°32′S, 27°0′E, *Richards 4813* (K). **Luapula:** Mweru Lake, near Nchelengi, 8°45′S, 29°0′E, *Bullock 3826* (K). **Lusaka:** Mazabuka, 4 mi. from Chirunda on Lusaka Road, 15°58′S, 28°48′E, *Drummond 5503* (K). **Northern:** Abercorn, Cassawa Sands, 8°30′S, 31°17′E, *Richards 10921* (K). **Southern:** Livingstone, 17°45′S, 25°52′E, *Rogers 7023* (K). **Western:** Barotse Floodplains, Lizulu Island, 15°30′S, 23°30′E, *Verboom 1039* (K). ZIMBABWE. **Binga District:** Mwenda Research Station, *Grosvenor 144* (K). **Manicaland:** Umtali, Zimunya's Reserve, 18°50′S, 32°37′E, *Chase 6853* (K). **Mashonaland North:** 20 km from Makuti on road to Kariba, Urungwe National Park, 16°50′S, 28°0′E, *Philcox & Leppard 8718* (K). **Mashonaland South:** 311.5 km from Harare towards Chirundu, Zambezi Escarpment, 17°27′S, 30°19′E, *Philcox & Leppard 8575* (K). **Matabeleland North:** 30 km from Makuti on road to Kariba, 17°0′S, 28°0′E, *Philcox et al. 8748* (K). **Matabeleland South:** tributary of Nuanetsi River, near Mabalauta, Gona Game Reserve, 21°10′S, 30°15′E, *Ngoni 160* (K).

Host-specific strains of *S. gesnerioides* differ in succulence, number of branches, pubescence and color of stem, bract length relative to calyx, length of calyx lobes relative to tube, and corolla color, pubescence, and size. Seed size is fairly uniform, 0.30 × 0.20 mm, for all strains of *S. gesnerioides* except for the *Euphorbia*-strain, which measures 0.39 × 0.24 mm.

Strains of *Striga gesnerioides*

While *S. gesnerioides* has evolved a strong host-specificity among its populations, these "morphs" do not have enough morphological differences to justify according them the level of species or subspecies (Mohamed, 1994). However, because of the potential for damage to crops and as a reference for further molecular work, we consider it important to record information on the host strains.

***Euphorbia* (Euphorbiaceae) Strain:** Perennial, succulent, 12–25 cm tall, with 2 to 4 branches from base. Stem greenish yellow, drying brown, terete, thick, densely pilose with retrorse hairs. Internodes 1–3.5 cm long. All bracts similar, lanceolate, ciliate, incurved, shorter than calyx. Flowers opposite/subopposite in dense spikes, spike much longer than vegetative stem. Calyx 7–9 mm long, pubescent; lobes unequal, lanceolate, ciliate, ½ the tube length. Corolla light blue or dark purple, tube 10–12 mm long, sparsely hairy, bent and expanded distally above calyx; lobes of lower lip obovate, obtuse; upper lip indistinctly bilobed, wider than long. Parasitic on arborescent *Euphorbia* species, i.e., *E. abyssinica* J. F. Gmel. (Musselman & Hepper, 1986).

Selected specimens examined. ETHIOPIA. **Welo:** 1 km NE of Desse, near Borkenna River, 11°7′N, 39°38′E, *Sutherland 381* (MO). NAMIBIA. **Namaland:** Great Namaqualand, Great Karas Mountains, 25°10′S, 17°50′E, *Ortendahl 487* (UPS). SOUTH AFRICA. **Cape:** Bathurst, bank of Kareiga River, 32°15′S, 23°30′E, *Bayliss 5212* (GH). **Transvaal:** Albany, Douglas Heights, 26°12′S, 28°15′E, *Bayliss 3158* (GH). SUDAN. **Red Sea:** Gabal Mawinja, *Musselman 6180* (ODU).

***Indigofera* (Fabaceae) Strain:** Plant annual, succulent, up to 16 cm tall, with 2 to 4 branches from base. Stem light green or purple, drying black, slender, obtusely square, furrowed, covered with dense, soft, divergent hairs. Internodes short, not exceeding 1 cm. All bracts similar, ciliate, shorter than calyx. Flowers opposite/subopposite in open spikes, spike longer than the vegetative stem. Calyx 5–7 mm long, ribs hispid, translucent between ribs; lobes equal or subequal, linear to lanceolate, ciliate, about the same length as tube. Corolla light pink to deep purple, tube 7–10 mm long, sparsely hairy, bent proximally within calyx teeth; lobes of lower lip obovate, obtuse, longitudinally rolled inside when dry; upper lip emarginate, as long as wide.

Selected specimen examined. SUDAN. **Blue Nile:** Abu Naama, Gabal Obeid, 12°15′N, 33°28′E, *Mohamed* 10/25/88 (ODU).

***Tephrosia* (Fabaceae) Strain:** Plant annual, up to 23 cm tall, unbranched. Stem pale green, often red, drying brown, slender, obtusely square, furrowed, glabrate. Internodes 2.5 mm long. All bracts similar, lanceolate, ciliate, much shorter than calyx. Flowers opposite/subopposite forming somewhat open spikes, spike much longer than vegetative stem. Calyx 5–6.5 mm long, ribs hispid, translucent between ribs; lobes unequal, lanceolate, ciliate, < ½ tube length. Corolla pink to light purple, tube 8–10 mm long, sparsely pubescent, bent and expanded distally above calyx, lobes of lower lip linear-oval; upper lip emarginate, wider than long.

Selected specimens examined. BURKINA FASO. **Center East:** 5 km N of Tenkodogo, 11°50′N, 0°19′W, *Safa & Musselman* 10/9/87 (ODU). CAMEROON. **Northern:** 29 km S of Garoua, 9°5′N, 13°25′E, *Musselman et al.* 10/16/88 (ODU). NIGERIA. **Niger:** Mokwa, 9°18′N, 5°2′E, *Knepper* 9/19/89 (ODU).

***Vigna* (Fabaceae) Strain:** Plant annual, succulent, 8–26 cm tall, branched from base. Stem green or purple, drying black, slender, obtusely square, furrowed, covered with sparse, soft, divergent hairs. Internodes 1–3 cm long. Bracts lanceolate, ciliate, incurved, lower ones as long as or longer than calyx, upper ones shorter than calyx. Flowers opposite forming somewhat dense spikes, spike usually shorter than vegetative stem. Calyx 6–9 mm long, ribs hispid, translucent between ribs; lobes unequal, acuminate, ciliate, ½ tube length. Corolla light pink or bluish, tube 8–10 mm long, glabrate, bent and expanded distally above calyx, lobes of lower lip obovate, spreading, slightly rolled inside; upper lip emarginate, as long as wide. Economically, this is the most important strain of *S. gesnerioides* because it attacks *Vigna unguiculata* (L.) Walp., cowpea, which is a staple in parts of Africa. Damage can be extensive to this and a few other crops (Parker & Riches, 1993).

Selected specimens examined. BURKINA FASO. **Northern:** 5 km S Gourcy, 13°9′N, 2°22′W, *Safa & Musselman* 10/10/87 (ODU). MALI. **Koulikoro:** Bamako, Sotuba, 12°50′N, 8°0′W, *Musselman & Mohamed* 10/1/88 (ODU). NIGER. **Maradi:** Maradi, Weed Research Organization Station, 13°32′N, 7°5′E, *Parker 2264* (ODU). NIGERIA. **Kano:** near Kano Airport, 12°5′N, 8°35′E, *Knepper* 2/22/89, 8/24/89 (ODU).

***Ipomoea* (Convolvulaceae) Strain:** Plant annual, succulent, 15–30 cm tall, usually unbranched. Stem purple, drying black, obtusely square, furrowed, somewhat thick, scabrous, very sparsely pilose, with soft, divergent, short hairs. Internodes 1–4 cm long. All bracts similar, slightly incurved, shorter than calyx. Flowers opposite in open spikes, spike as long as or longer than vegetative stem. Calyx 5–7 mm long, ribs hispid, translucent between ribs; lobes unequal, acuminate, ciliate, shorter than tube. Corolla white to light blue, tube 9–13 mm long, glabrate, bent and expanded distally above calyx, lobes of lower lip spreading, linear-oval; upper lip emarginate, about as long as wide.

Selected specimens examined. MALI. **Koulikoro:** 14 km N Koulokani, 13°38′N, 7°54′W, *Musselman & Mohamed* 10/6/88 (ODU). SENEGAL. **Dakar:** 10 km N Dakar, *Musselman et al. 7066* (ODU). **Diourbel:** Kheur Serigne Sarr, 16 km E Diourbel, 14°39′N, 16°0′W, *Musselman 7064* (ODU). SUDAN. **South Kordofan:** Balangya, 10°30′N, 29°48′E, *Musselman* 8/23/86 (ODU).

***Jaquemontia* (Convolvulaceae) Strain:** Plant annual, succulent, up to 30 cm tall, unbranched or with 2–3 branches from base. Stem purple, drying black, slender, obtusely square, furrowed, sparsely to densely pilose with divergent hairs. Internodes 2–4 cm long. All bracts similar, incurved, ciliate, shorter than calyx. Flowers opposite in open spikes, spike very much longer than vegetative stem. Calyx 5–8 mm long, ribs hispid, translucent between ribs; lobes unequal, acuminate, < ½ tube length. Corolla pale blue or mauve, tube 8–11 cm long, glabrate, bent and expanded distally above calyx, lobes of lower lip obovate; upper lip widely obovate, indistinctly bilobed, wider than long.

Selected specimens examined. NIGER. **Dosso:** Kane, 224 km from Niamey on Maradi Road, 13°26′N, 3°53′E, *Parker 2312* (ODU). **Maradi:** Maradi, 13°32′N, 7°5′E, *Parker 2031* (K, ODU). NIGERIA. **Plateau:** Jos–Shendam Road, 9°50′N, 9°25′E, *Knepper* 8/26/89 (ODU). SOUTH AFRICA. *Musselman 6269* (ODU).

***Merremia* (Convolvulaceae) Strain:** Plant annual, succulent, up to 18 cm tall, unbranched or with 2–3 branches from base. Stem purple, drying black, slender, obtusely square, furrowed, sparsely to densely pilose, with divergent hairs. Internodes short, 2 cm long. All bracts similar, ciliate, incurved, shorter than calyx. Flowers imbricate forming dense spikes, spike very much longer than vegetative stem. Calyx 5–6 mm long, ribs hispid, translucent between ribs; lobes unequal, acuminate, about ½ tube length. Corolla purple, tube 8–10 mm long, glabrate, bent and expanded distally above calyx, lobes of lower lip linear-obovate, longitudinally rolled inside when dry; upper lip emarginate, wider than long.

Selected specimens examined. SUDAN. **Bahr-Al-Ghazal:** Djur River, *Aymlar* 27/51 (K). SUDAN. **Blue Nile:** Tozi, 12°54′N, 33°36′E, *Mohamed* 10/26/88 (ODU).

***Nicotiana* (Solanaceae) Strain:** Plant annual, 15–22 cm tall, unbranched. Stem pale green, drying brown, slender, obtusely square, furrowed, covered with dense, short, soft, divergent hairs. Internodes 1–3 cm long. Bracts ciliate, incurved, lower ones as long as or longer than calyx, upper ones shorter than calyx. Flowers sparse, opposite in open spikes, spike usually shorter than vegetative stem. Calyx 4–7 mm long, ribs hispid, translucent between ribs; lobes unequal, acuminate, about ½ tube length. Corolla pink/mauve, tube 8–10 mm long, with few hairs or glandular hairs, bent and expanded distally above calyx, lobes of lower lip obovate; upper lip emarginate, as long as wide. Tobacco in Zimbabwe has been seriously damaged by this strain (Wild, 1948).

Selected specimens examined. NIGER. **Maradi:** Maradi, near the Weed Research Organization Station, 13°32′N, 7°5′E, *Parker 1316, 2265* (ODU).

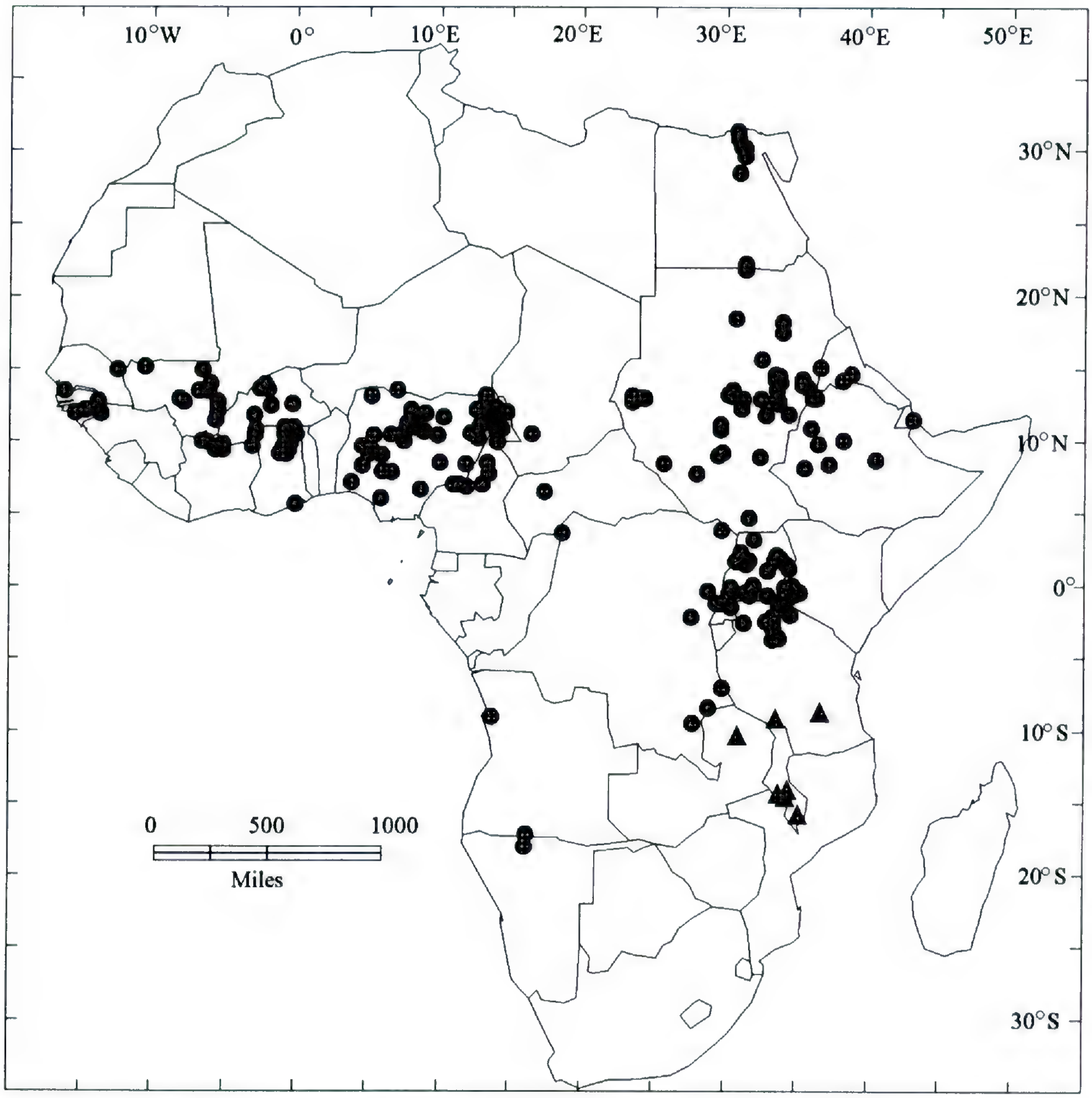


Figure 10. Distribution based on specimens of *Striga gracillima* (triangle) and *S. hermonthica* (circle).

14. Striga gracillima H. Melchior, in Mildbraed, Notizbl. Bot. Gart. Berlin-Dahlem 11: 681–682. 1932. TYPE: Nyassa Hochland. Station Kyimbila, *Stolz 1304* (holotype, B?; isotypes, BM!, K!).

Annual, 25–55 cm tall, slender, usually unbranched, densely scabrous, hispid, hairs ascending, stem obtusely square. Leaves 18–45 × 2–5 mm, opposite, narrowly elliptic, longer than internodes, margin entire, veins obscure. Lower bracts 10–25 × 2 mm, leaf-like, longer than calyx, upper bracts lanceolate, as long as or just shorter than calyx. Flowers alternate, shortly pedicellate, axillary at the base of inflorescence, becoming imbricate above middle, forming strobilus-like inflorescences, raceme shorter than vegetative stem. Calyx 5-ribbed, 5–7 mm long; tube 3–4(–5) mm long; lobes 5, unequal (adaxial reduced to < 1 mm), deltate, 1–2 mm long, shorter than tube. Corolla purple, tube 6–8 mm long, bent and expanded proximally within calyx teeth, densely hairy, never glandular-pubescent; lobes of lower lip 1–3 × 1–1.5 mm, spreading, obovate, upper lip 4 × 5 mm, obovate, emarginate, as long as wide.

Striga gracillima is a poorly understood species and has mistakenly been identified as *S. aspera* (Hepper, 1984). It has been collected from Malawi, Tanzania, and Zambia between 8°41′S and 15°45′ S (Fig. 10). *Striga gracillima* differs from *S. hermonthica* in having a slender, sparsely branched stem; narrower, erect, linear-elliptic leaves; shorter corolla tube with smaller lobes; and much smaller calyces than those of *S. hermonthica*. The glandular pubescent corolla and the equal calyx lobes are among the features that distinguish *S. aspera* from *S. hermonthica* and *S. gracillima*. *Striga gracillima* has not been reported to attack crops. Its host range has not been investigated.

Selected specimens examined. MALAWI. **Central:** Dedza, Dedza Mountain Forest, 14°22′S, 34°20′E, *Salubeni 1330* (GH, K, MO); Dedza, near Kanjali, Chongoni Forest, 14°27′S, 34°18′E, *Salubeni 1341, 1539* (K); Lilongwe, 25 mi. S of Lilongwe, 14°17′S, 33°47′E, *Pawek 14332A* (K); Kyimbila, *Stolz 1304* (GH, MO); Nyassa Hochland, Station Kyimbila, *Stolz 1304* (BM, K). **Southern:** Chiradzulu, Chiradzulu Mountain, 15°45′S, 34°18′E, *Brummit & Banda 9838* (K). TANZANIA. **Morogoro:** Mahenge, 8°41′S, 36°41′E, *Schlieben 2108* (US). ZAMBIA. **Northern:** Kasama, 40 km W of Kasama, 10°16′S, 30°54′E, *Philcox et al. 10210* (K).

15. Striga hallaei A. Raynal, Bull. Jard. Bot. Belg. 39: 374. 1969. TYPE: Gabon. Efout, *Halle & Villiers 4914* (holotype, P!).

Annual, 30–60 cm tall, erect, slender, unbranched, pilose, stem obtusely square. Leaves 40–70 × 12(–30) mm, opposite, elliptic, shortly petiolate, coarsely toothed, 3-veined, shorter than internodes. Lower bracts 8–20 × 5 mm, leaf-like, upper ones reduced, all bracts longer than calyx. Flowers alternate in open racemes, raceme shorter than vegetative stem. Calyx 5-ribbed, 6–7 mm long; tube 2 mm long; lobes 5, equal, linear to lanceolate, 4–5 mm long, twice as long as tube. Corolla salmon-pink, tube 22–25 mm long, bent and expanded distally above calyx, densely pilose; lobes of lower lip 17 × 30 mm, broadly round; upper lip 12 × 15 mm, emarginate, reflexed backward.

This species inhabits openings within rainforests (Raynal-Roques, 1969) of Gabon and Democratic Republic of Congo (Fig. 7). The large toothed and nerved leaves, long calyx lobes, salmon-pink corollas, and adaptation to wet habitats suggest a relationship between this species, *S. forbesii,* and *S. latericea.* Of these three species, only *S. hallaei* has a 5-ribbed calyx.

16. Striga hermonthica (Del.) Benth., Companion Bot. Mag. 1: 365. 1836. *Buchnera hermonthica* Del., Fl. Egypte: 245, 34 fig. 3. 1813. TYPE: Egypt. *Delile s.n.* (holotype, P!).

Striga senegalensis Benth., in DC. Prodr. 10: 502 (1846). TYPE: Senegal. *Heudelot s.n.* (syntype, K!).

Annual, to 90 cm tall, stiffly erect, branched from middle, scabrous, densely hispid, hairs ascending, stem square, furrowed. Leaves 15–80 (–110) × (4–)10–25(–40) mm, opposite, linear or narrowly elliptic, ascending or spreading, longer than internodes, margin entire, veins obscure. Lower bracts 12–50(–95) × 2–5(–10) mm, leaf-like, longer than calyx, upper bracts lanceolate, as long as or shorter than calyx. Flowers opposite, forming a lax spike at base of inflorescence, becoming denser above middle, spike shorter than vegetative stem. Calyx 5-ribbed, 7–12 mm long; tube 5–10 mm long; lobes 5, unequal (adaxial reduced to < 1 mm), deltate, 2–4 mm long, shorter than tube. Corolla pink or mauve, rarely white, with ascending hairs, tube (13)16–20 mm long, bent and expanded proximally within calyx teeth, sparsely hairy, never glandular-pubescent; lobes of lower lip 7–15 × 3–6(–10) mm, obovate, upper lip 4–9 × 4–7 mm, emarginate.

Striga hermonthica, giant witchweed, is a serious pest of food crops (sorghum, maize, millet, rice) especially in the Sahel of Africa. In fact, in parts of the Sahel it causes more damage to millet and sorghum than any other factor, with the possible exception of birds. *Striga hermonthica* is not particular to soil type or pH and can be abundant on lighter soils in West Africa, as well as on heavy clay soils in East Africa.

Giant witchweed is an obligate outcrosser as is the related species, *S. aspera* (Musselman et al., 1991). Several insects are potential pollen vectors. Comparison of populations of *S. hermonthica* parasitizing similar hosts but growing in different geographical localities revealed a high level of morphological and floral variability. This high phenetic variability was accompanied by high genetic diversity (Bharathalakshmi et al., 1990; Olivier et al., 1998).

The sorghum- and millet-strains of *S. hermonthica* have similar vegetative characteristics but different corolla sizes and shades. The millet-strain has a smaller dull pink corolla and is common on sandy soil, whereas the sorghum-strain has a larger pink corolla and is associated with clay soils. Although host-specificity reduces the host range of the parasite, it may lead to successful attachment and better adaptability to a given host under specific conditions. Such floral differences could be expected to reduce cross pollination between the two strains when sympatric. Breeders developing grains for tolerance to the parasite need to be aware of genetic interchange in this wide-ranging species.

Striga hermonthica extends from Senegal to Ethiopia with southern limits in the Democratic Republic of Congo and Tanzania, between 22°3′N and 9°30′S (Fig. 10). There are a few collections from Angola and Namibia. Collections from the Nile Delta and parts of Yemen likely represent introductions with contaminated seed, a well-documented means of dispersal (Berner et al., 1992). *Striga hermonthica* has not been found in native grasslands except in the Nuba Mountains of the Sudan Republic (Musselman & Hepper, 1986).

South of 9°30′N, *S. hermonthica* is less frequent and smaller in size. *Striga hermonthica, S. asiatica,* and *S. gesnerioides* are the only species found north of the Sahara (Figs. 4, 8, 10).

In any large population of *S. hermonthica* it is possible to find albino plants. These are plants totally lacking chlorophyll yet capable of flowering because of their parasitic nature (Musselman & Hepper, 1986).

Selected specimens examined. ANGOLA. **Luanda:** Catete, 9°2′S, 13°42′E, *Gossweller 199, 14219* (K). BURKINA FASO. **Central:** 60 km E of Ouagadougou, 12°35′N, 0°18′W, *Safa & Musselman* 10/9/87 (ODU). **Northern:** Bogoya, 5 km NW of Ouahigouya, 13°38′N, 2°27′W, *Raynal 22263* (K). **Volta:** Boromo, 11°50′N, 2°55′W, *Raynal 22600* (K). CAMEROON. **Center South:** near Djikainke, 4 mi. from Foumban, 4°43′N, 10°55′E, *Leeuwenberg 10189* (K). **Northern:** Adamawa, near Gangumi, 7°0′N, 13°0′E, *Latilo & Daramola 28792* (K). CENTRAL AFRICAN REPUBLIC. **Ouham:** Bossango Region, 6°30′N, 17°20′E, *Fay & Doka 5070* (K). DEMOCRATIC REPUBLIC OF CONGO. **Equateur:** Libenge, 3°39′N, 18°38′E, *Lebrun 1759* (GH). **Haba:** Niabembe, 2°14′S, 27°44′E, *Steyagi s.n.* (US). **Haut Zaire:** between Mahagi and Mahagi Port, 2°16′N, 31°7′E, *Boutique 308* (K). **Kivu:** between Gabiro and Kidebe, 1°30′S, 30°25′E, *Lebrun 9631* (K). **Shaba:** Kundelungu, 9°30′S, 27°45′E, *Kassner exped. 2786* (K). DJIBOUTI. **Eastern:** Arta Road, 11°32′N, 43°9′E, *Curle 98* (K). EGYPT. **Cairo:** S of Al-Jizah, Helwan, 29°58′N, 31°16′E, *Boland 743* (K). **Delta:** Rodah, 30°15′N, 31°15′E, *Schweinfurth 1880* (US). **Red Sea:** Al-Fashn, 28°27′N, 30°55′E, *Faad 1881* (K), *Radjil 594* (GH). ERITREA. **Southern:** 4 km S of Adi Kuala, 14°38′N, 38°50′E, *Ryding et al. 1548* (K). **Teseney:** 15°6′N, 36°40′E, *Pichi-Sermolli 1295* (K). ETHIOPIA. **Gojam:** Shoa, 10°5′N, 38°16′E, *Meyer s.n.* (K). **Ilubabor:** Blue Nile, Gore, 8°9′N, 35°32′E, *Parker E22* (K). **Kefa:** Omo National Park, 8°25′N, 37°15′E, *Ash 3648* (K). **Ogaden Harerge:** at 8°44′N, 40°30′E, *Puff et al. 820922* (K). **Shewa:** N Addis Ababa, 9°12′N, 38°42′E, *Meyer 7594* (US). **Tigray:** Enda Sellassie, W of Axum, 14°10′N, 38°11′E, *Scott 217* (K). **Welega:** Gondor, 9°50′N, 36°31′E, *Massey 47* (K). GAMBIA. **Central:** Sapu, 13°0′N, 15°0′W, *Terry s.n.* (K), *Saunders 60, 62* (K), *Brookes 19* (K). GHANA. **Northern:** 2.3 mi. NW of Bunkpurugu, 10°32′N, 0°3′E, *Innes 30748* (K). **Upper:** Bongo, near Bolgatanga, 10°50′N, 0°50′W, *Hossain & Agyakwah 37855* (K). GUINEA BISSAU. **Central:** Bafata, Arredores Region, 12°12′N, 14°42′W, *Espirito Santo 2796* (K). **Eastern:** Gahee, Canquelifa, 12°40′N, 13°50′W, *Espirito Santo 2960* (K). IVORY COAST. **Bouna:** Varale, 60 km N of Bouna, 9°39′N, 3°6′W, *Geerling & Bokdam 934* (K). **Boundiali:** Zaguinaso, 64 km N of Boundiali, 10°2′N, 6°32′W, *Riches 249* (K). **Firkessedougou:** 5 km S of Firkessedougou, 9°36′N, 5°8′W, *Riches 226* (K). KENYA. **Nyanza:** 3 mi. S of Homa Bay, 0°34′S, 34°22′E, *Bogdan 4535* (K). MALI. **Kayes:** Dionkoulane Fields, Yelimane Circle, 15°8′N, 10°34′W, *Yeafes 88* (K). **Koulikoro:** Sekesona, 7 km from Ouelessebougou, 90 km S of Bamako, *Raynal 22194* (K). **Segou:** 7 km from Diabaly, 14°0′N, 6°0′W, *Raynal 22573* (K). **Sikasso:** 10 km W of Yangasso, *Raynal 22221* (K). NAMIBIA. **Cunene:** Oshikango, 17°9′S, 16°10′E, *Rodin 9379* (K). **Owambo:** Olukonda, 18°0′S, 16°3′E, *Reed 14* (K). NIGER. **Maradi:** 2 km W Maradi on Niamey Road, 13°32′N, 7°4′E, *Parker 2316, 2317* (ODU). **Niamey:** Niamey, Galome, 13°35′N, 2°10′W, *Hagerup 478* (K), *Barter 136, 1821* (GH). NIGERIA. **Bauchi:** 1 mi. N of Naraguta, 10°40′N, 8°55′E, *Hepper 1162* (K). **Bornu:** Biu, 10°35′N, 12°10′E, *Noble 30, 31* (K). **Gongola:** 10 mi. W of Gombi, 10°12′N, 12°41′E, *Lowe 1671* (K). **Kaduna:** between Zaria and Kano, site of new tollgate, 11°30′N, 8°10′E, *Lowe 4828* (K). **Kano:** Gaya, near Kano, 11°55′N, 9°2′E, *Shorland 179* (K). **Kwara:** Lokoja, 7°50′N, 6°40′E, *Parsons L79* (K). **Niger:** Kontajora, 10°25′N, 5°25′E, *Dalziel 171* (K). **Ogun:** Ibadan, Botany Department, 7°10′N, 3°50′E, *Lowe 2760* (K). **Plateau:** Jos-Shendam Road, 9°50′N, 9°25′E, *Knepper 8/26/89* (ODU). **Sokoto:** Guadabawa, 13°8′N, 5°15′E, *Ryan 58* (K). SENEGAL. **Oriental:** Bakel, 14°55′N, 12°29′W, *Hepper 3721* (K). SUDAN. **Al-Jazirah:** Madani, Barakat, 14°20′N, 33°48′E, *Musselman 6139* (ODU, K). **Bahr Al-Ghazal:** Wau, 7°46′N, 28°2′E, *MacCintosh 103* (K). **Blue Nile:** Abu Naama, 12°49′N, 33°40′E, *Mohamed* 10/27/88 (ODU). **Kassala:** Al-Qadarif, 14°4′N, 35°24′E, *Schweinfurth 595* (K). **Khartoum:** at 15°38′N, 32°32′E, *Sawer 13698, 13699* (K). **North Darfur:** Gabal Kurdufan, 13°23′N, 23°32′E, *Wickens 628* (K). Gabal Abu Sinun, 12°52′N, 23°30′E, *Wickens 309* (K). **North Kurdufan:** El Obeid, Khartaget, 13°13′N, 30°32′E, *Musselman 6162* (ODU). **Northern:** Wadi Halfa, 1 km NW Debeira Station, 22°3′N, 31°23′E, *Petterson 16819* (K). **South Kurdufan:** near Kadugli, 11°1′N, 29°41′E, *Musselman 6155* (K). **Upper Nile:** Sobat, 8°55′N, 32°25′E, *Petherick 1863* (K). **West Kurdufan:** El Greo, *Pfund 679* (K). TANZANIA. **Mara:** Lake Province, Ikoma, Musoma, 2°2′S, 34°35′E, *Tanner 4142* (K). **Shinyanga:** near Shinyanga, 3°40′S, 33°27′E, *Box 293* (K). **Ziwa Magharibi:** West Lake Province, Biharamulo, 2°37′S, 31°19′E, *Tanner 5274* (K). CHAD. **Chari-Baguirmi:** Ba Ill, 10°32′N, 16°27′E, *Audry B2* (K). TOGO. **Northern:** 7 km N of Mango, 10°26′N, 0°17′E, *Pash 7916* (K). UGANDA. **Eastern:** Amuria, 2°4′N, 33°37′E, *Willemse 30* (K). **North Buganda:** Sempayo–Kiboga Road, Semlilci Flats, 0°40′N, 31°45′E, *Liebenberg 1029* (K). **Northern:** between Nimule and Gondokoro, along Bahr El Gabal, 3°10′N, 32°5′E, *Mearns 3005* (GH). **Southern:** W Ankole, 0°30′S, 30°18′E, *Dawe 444* (K). **Western:** Bunyoro, 1°38′N, 31°30′E, *Fyffe 32, 164* (K).

17. Striga hirsuta Benth., in DC. Prodr. 10: 502. 1846. TYPE: China. *Loureiro s.n.* (holotype, P!). Figure 2.

Striga pusilla Hochst, in DC. Prodr. 10: 503. 1846. TYPE: Ethiopia. Gafata, *Schimper 1209* (holotype, K!).

Annual, small, 2–6(–20) cm tall, slender, unbranched or branched from middle, sparsely hispid to ciliate, hairs ascending, stem obtusely square, furrowed. Leaves reduced, 3–15 × 0.5–1 mm, opposite/subopposite, linear, shorter than internodes, margin entire, veins obscure. Lower bracts 6–15 × 1 mm, narrowly lanceolate, longer than calyx, upper bracts lanceolate, shorter than calyx. Lower surface of leaves and bracts with a row of hispid hairs along margins and midrib, upper surface hispid only along margins. Flowers alternate in open racemes, raceme longer than vegetative stem. Calyx

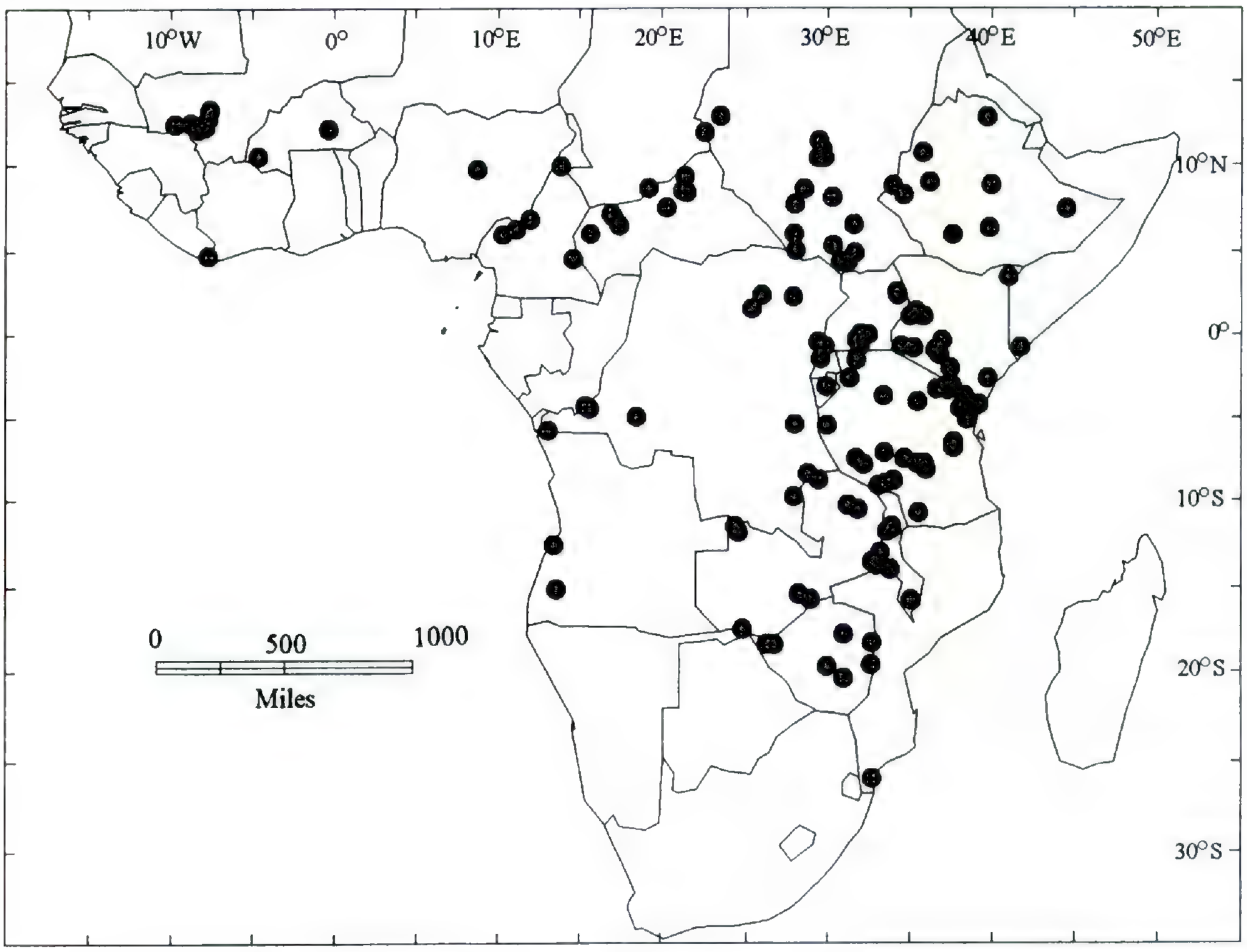


Figure 11. Distribution based on specimens of *Striga hirsuta* (circle).

10-ribbed, 4–5.5 mm long; tube 2–3 mm long; lobes 5, equal, subulate, 1.5–2.5 mm long, as long as tube. Corolla dark red with yellow throat, 8–10 (–12) mm long, bent and expanded distally above calyx, sparsely glandular-pubescent; lobes of lower lip 2–3(–5) × 1–2(–3) mm, obovate, upper lip 1–2 × 2–4 mm, emarginate.

Striga hirsuta is frequent in the Sudanian savanna. Its range extends from Mali to Ethiopia and south into Kenya, Tanzania, Malawi, and Mozambique between 13°N and 26°S (Fig. 11). *Striga hirsuta* and *S. lutea* may rarely attack crop plants such as sorghum and maize, but no noticeable damage occurs.

Striga hirsuta is typically a small plant, up to 6 cm, that emerges, flowers, and matures in a fairly short time. It flowers sooner after the commencement of the rains than the most damaging species, *S. hermonthica*. *Striga hirsuta* is less hispid than *S. asiatica* and *S. lutea*; however, it is more scabrous than both species. See comments under *S. asiatica*. Musselman and Hepper (1986) included *S. hirsuta* and *S. lutea* in *S. asiatica*. Further work is needed to elucidate the relationship of this species to *S. asiatica*.

Selected specimens examined. ANGOLA. **Benguella:** Benguella, 12°36′S, 13°20′E, *Gossweller 2916* (K). **Huila:** Couceiro, Vila Parire, 15°10′S, 13°30′E, *Henriques 853* (K). BURKINA FASO. **Eastern:** Fada-N'rGourma, 12°12′N, 0°25′E, *Safa & Musselman* 10/25/87 (ODU). BURUNDI. **Northern:** Bubanza, Imbo, 3°10′S, 29°58′E, *Reekmans 10314* (K). CAMEROON. **Eastern:** Batouri, 4°30′N, 14°30′E, *Taylor 1* (K). **North-Western:** Nanpe, 8 km W of Bangante, 8°5′N, 10°36′E, *de Wilde & de Wilde-Duyfjes 2397* (K). **Northern:** Jangla, foot of Vogel Peak, 8°22′N, 11°55′E, *Hepper 2745* (K). **Western:** Mbaw Plain, 6°15′N, 11°0′E, *Brunt 404* (K). CENTRAL AFRICAN REPUBLIC. **Bamingui-Bangoran:** 2 km E of Camp Koumbala, 8°29′N, 21°24′E, *Fay 2867* (K). **Nana-Mambere:** Bouar, 6°0′N, 15°35′E, *Mildraed 9501* (K). **Ouham-Pende:** near Nana-Bakassa River, 7°0′N, 17°0′E, *Fay 5068* (K). CONGO. **Kinshasa:** at 4°30′S, 15°30′E, *Woolhouse 51/61/22* (K). DEMOCRATIC REPUBLIC OF CONGO. **Bas-Zaire:** Boma, 5°48′S, 13°0′E, *Dacremont 128, 129* (K). **Haut Zaire:** Ibila-Bofea, 2°20′N, 26°0′E, *Eviard 2231* (K). **Katanga:** Kayuba, 9°45′S, 27°58′E, *de Witte 3332* (K). **Kivu:** Beni, 0°30′N, 29°28′E, *Gille 39* (K). **Shaba:** Pweto, 8°25′S, 28°51′E, *Robyn 1971* (K). ETHIOPIA. **Bale:** Dallo, 6°22′N, 39°50′E, *Mooney 8427* (K). **Gamo Gofa:** 8 km S of Arba Minch, 5°58′N, 37°36′E, *Gilbert et al. 320* (K). **Gojam:** Beles Farm, W of Gojam, 10°50′N, 35°47′E, *Parker 4074* (K). **Ilubabor:** Gambela, 8°18′N, 34°37′E, *Ash 518* (K). **Ogaden Harerge:** Awash National Park, 8°55′N, 39°55′E, *Gilbert 1663* (K). **Tigray:** Gafata, 12°55′N, 39°40′E, *Anonymous 1842* (GH, US). **Welega:** 9°3′N, 36°11′E, *Gilbert & Thulin 708* (K). KENYA. **Central:** Zawadi, 0°24′S, 36°59′E, *Faden et al. 74/572* (K). **Coast:** 30 km W of Buru, 3°45′S, 38°18′E, *Durham* 26/2/24 (K). **Eastern:** Emali Hill, 2°3′S, 37°27′E, *Someren 250* (K).

Nairobi: 1°17′S, 36°48′E, *Johnstone 1551* (K), *Red 1424* (K). **Rift Valley:** 2 km S of Lake Naivasha, 0°50′S, 36°14′E, *Gilbert 4840* (K). LIBERIA. **Maryland:** Harper, 4°25′N, 7°43′W, *Baldwin 5988* (US). MALAWI. **Central:** Fort Manning, Bua River, 13°47′S, 32°58′E, *Robson 1083* (K). **Northern:** 37 mi. SW Mzuzu, 11°47′S, 33°38′E, *Pawek 10942* (K). **Southern:** Blantyre, 2 mi. N of Limbe, 15°49′S, 35°7′E, *Brummit 8428* (K). MALI. **Koulikoro:** Baguineda, 22 km N of Bamako, 12°51′N, 7°46′W, *Musselman & Mohamed* 10/8/88 (ODU). MOZAMBIQUE. **Maputo:** Inhaca Island, 23 km E of Maputo (Lourenco Marques), 26°1′S, 32°57′E, *Mogg 30317* (K). NIGER. *Barter 530* (ODU). NIGERIA. **Plateau:** Jos-Plateau, 9°50′N, 8°40′E, *Knepper* 8/25/89 (ODU). RWANDA. **Kaduna:** Tegina, *Musselman & Mansfield 5531* (ODU). **Northern:** Ruhengeri, 1°30′S, 29°36′E, *Reekmans 4257* (K). SEYCHELLES ISLAND. Vallee de Mai, *Belle 196* (GH). SOMALIA. **Coast:** 5 km S of Goba, 0°47′N, 41°41′E, *Gillett et al. 5045* (K). SUDAN. **Bahr Al-Ghazal:** Djur River, 8°40′N, 28°35′E, *Ayhuer GA27151* (K). **Darfur:** Kalwe, *Hynes 478* (K). **Equatoria:** Nakbi, between Yambio and Tambio, 5°1′N, 28°5′E, *Myers 7000* (K). **Jungali:** near Baidit, 45 km N of Bor, 6°32′N, 31°36′E, *Lock 81/247* (K). **North Darfur:** Gabal marra, Kibbi, 12°1′N, 23°32′E, *Wickens 2241* (K). **South Kurdufan:** between Belatama and Kadugli, 11°30′N, 29°30′E, *Sherif 3952* (K). **Upper Nile:** Adok, 8°10′N, 30°18′E, *Petherick 1862* (K). TANZANIA. **Arusha:** Mbulu, Jebogo Hill, Mgati Plains, 4°5′S, 35°30′E, *Burtt 2105* (K). **Iringa:** 80 km N of Muhange, 8°10′S, 36°0′E, *Bidgood et al. 65* (K). **Kigoma:** Livingstone College, 5°30′S, 30°0′E, *Verdcourt 3390* (K). **Kilamanjaro:** Ngungu, Shimba Hills, 4°28′S, 38°4′E, *Jackson 415* (K). **Mafia:** Chungaruma, *Greenway 5371* (K). **Magharibi:** Biharamulo, on Nyamirembe Road, 2°39′S, 31°23′E, *Balslev 447* (MO). **Mbeya:** Chimala, 85 km E of Mbeya, 8°52′S, 34°0′E, *Bally & Carter 16448* (K). **Morogoro:** 25 mi. NE of Morogoro, 6°30′S, 37°40′E, *Bidgood et al. 429* (K). **Rukwa:** Tumba, 7°30′S, 31°45′E, *Bullock B14* (K). **Ruvuma:** 10 km SW of Songea, 10°44′S, 35°33′E, *Milne-Redhead & Taylor 882* (K). **Shinyanga:** near Shinyanga, 3°45′S, 33°27′E, *Box 291* (K). **Tanga:** Handeni, Nyongolo Hill, 5°27′S, 38°2′E, *Mgaza 434* (K). **Zanzibar:** *Kirk 2179* (K). **Ziwa Magharibi:** Muhutwe Bukoba, 1°35′S, 31°45′E, *Panayotis 98* (K). UGANDA. 2 mi. S of Akilok, *Langdale-Brown 2421* (K). **Central:** Entebbe, 0°4′N, 32°28′E, *Brown 21, 238* (K). **Karamoja:** SE of Karamoja, NE of Kocemaluk, 2°18′N, 34°18′E, *Hudson 296* (K). **Nile:** Koboko, 3°25′N, 31°0′E, *Looe P1048* (K). **North Buganda:** 10 km NW of Katera, 0°46′N, 31°58′E, *Lye s.n.* (K). **South Buganda:** Lake Kauonje, Mountain Masak, 0°21′S, 31°45′E, *Rose 10253* (K). **Southern:** Kambugo, Kisizi, 0°plain 45′S, 29°50′E, *Purseglove P2424* (K). ZAMBIA. **Eastern:** Fort Jameson, 13°37′S, 32°44′E, *Bush 23* (K). **Lusaka:** 1.5 km N of Marangora, Zambezi Escarpment, Urungwe National Park, 15°45′S, 29°0′E, *Philcox et al. 8563, 8610* (K). **North-Western:** Mwinilunga, near Matonchi, 11°41′S, 24°24′E, *Townsend 170* (K). **Northern:** 16 km E of Kasama, Nambkungu Ridge, 10°16′S, 31°15′E, *Philcox et al. 10190* (K). **Western:** Sesheke, 17°30′S, 24°50′E, *Gairdner 412* (K). ZIMBABWE. **Inyanga:** Inyanga, 18°15′S, 32°42′E, *Norlindh & Weimarck 4956* (K). **Manicaland:** River Tandai, 19°32′S, 32°40′E, *Myres 294* (K). **Mashonaland South:** Salisbury Experimental Station, 17°48′S, 31°1′E, *Wild 5718* (K). **Matabeleland North:** near Wankie Prison, 18°22′S, 26°30′E, *Raymond 319* (K). **Matabeleland South:** Selukwe, near Surprise Mine, 19°38′S, 30°0′E, *Cecil 128* (K). **Victoria:** Kyle National Park, 20°19′S, 31°1′E, *Philcox & Leppard 8870* (K).

18. Striga junodii Schinz., Mem. Herb. Boiss 10: 62. 1896–1900. TYPE: Mozambique. Delagoa Bay, *Junod 183* (holotype, G not seen).

Perennial, 30–60 cm tall, stiffly erect, unbranched, scabrous, hispid, stem winged. Leaves 10–25 × 1–2 mm, linear, opposite at base of stem, alternate above, shorter than internodes, margin entire, veins obscure. Lower and upper bracts similar, 2–5(–12) × 1 mm, linear, shorter than calyx. Flowers alternate in open racemes, raceme much shorter than vegetative stem. Calyx 15–18-ribbed, 11–15 mm long; tube 7–9 mm long; lobes 5, equal, linear to lanceolate, 4–7 mm long, slightly shorter than tube. Corolla purple, tube 20–22 mm long, bent and expanded distally above calyx, densely pubescent; lobes of lower lip 8–11 × 4–5 mm, spreading, obovate, slightly notched; upper lip 5–8 × 8–11 mm, emarginate.

Striga junodii is represented by only a few collections from swampy grounds in southern Mozambique and northeastern South Africa between 24°S and 26°S (Fig. 12). It does not extend north into Mozambique where the similar *S. pubiflora* occurs (Fig. 15). The two species may have different ecological requirements. *Striga junodii* is also morphologically similar to the Asian species, *S. masuria*; however, *S. junodii* has a characteristic notch on the lower corolla lobes. The current status of *S. junodii* remains unknown. We are unaware of extant populations. Collectors in Mozambique and South Africa should be aware of its presence.

Selected specimens examined. MOZAMBIQUE. **Inhambane:** Inhambane, 23°54′S, 35°30′E, *Anonymous 12092* (MO, S, UPS). **Maputo:** Lourenco Marques, Rikatla, 25°58′S, 32°32′E, *Junod 276* (BRE). SOUTH AFRICA. **Transvaal:** Muzi Swamp, 2 km from Phelendaba Crossing on Mbazwana Road, 23°55′S, 31°13′E, *Germishuizen 3534* (K); Nelspruit, 25°30′S, 30°59′E, *Prosser 1784* (BRE, K), *Britton 4778* (BRE).

19. Striga klingii (Engl.) Skan, Fl. Trop. Afr. 4(2): 413. 1906. *Buchnera klingii* Engl., Bot. Jahrb. 18: 69. 1893. TYPE: Togo. *Büttner 239/221* (B?).

Annual, to 86 cm tall, coarse, stiffly erect, often 3-branched just below inflorescence, scabrous, hispid, stem obtusely square. Leaves 20–40(–70) × 4–7(–12) mm, opposite, narrowly elliptic, tips obtuse, 3-veined, coarsely toothed, shorter than internodes. Lower bracts 10–20 × 2–4 mm, lanceolate, longer than calyx, upper bracts lanceolate, as long

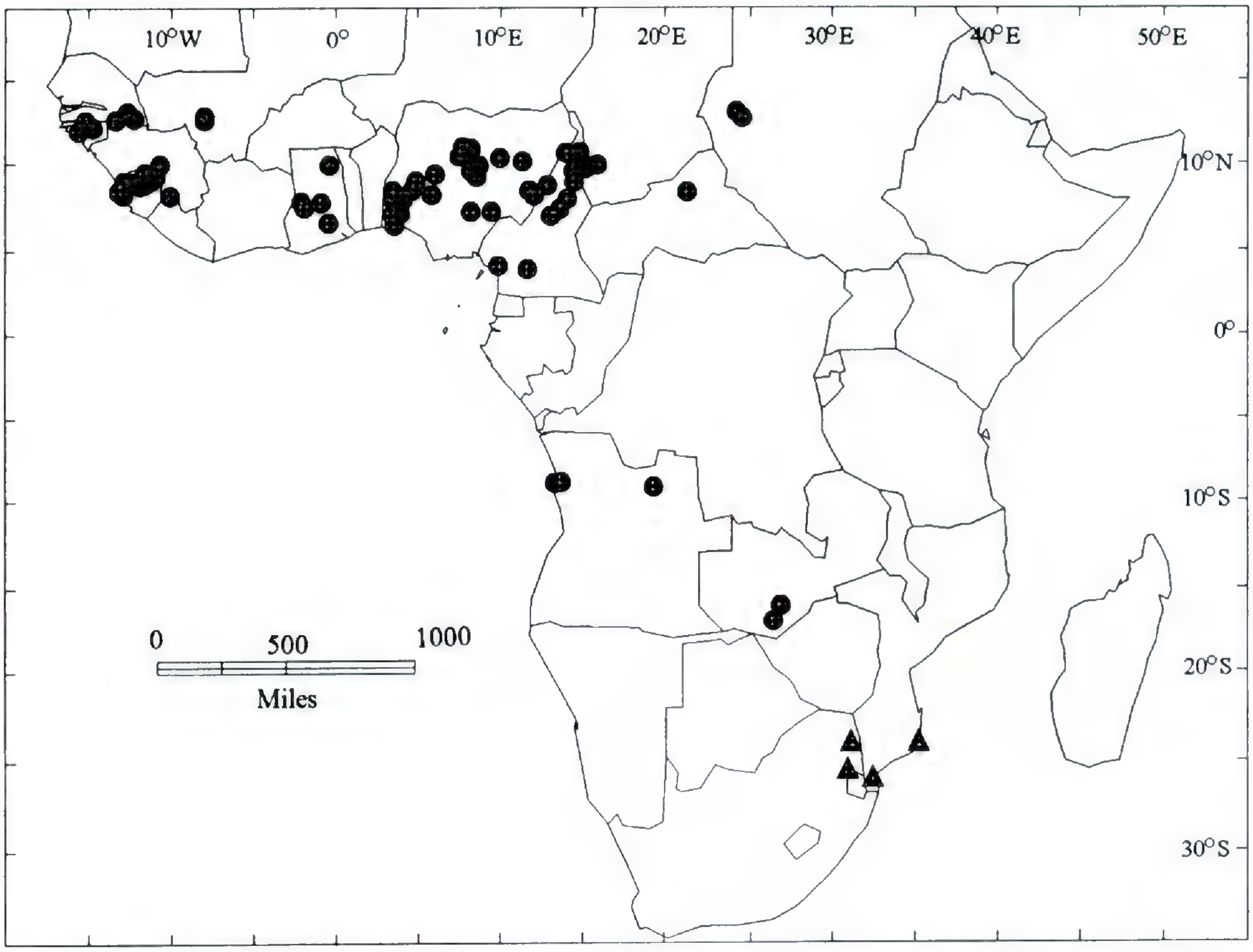


Figure 12. Distribution based on specimens of *Striga junodii* (triangle) and *S. macrantha* (circle).

as calyx. Flowers imbricate forming very compact spikes, spike much shorter than vegetative stem. Calyx 12–15-ribbed, 8–10 mm long; tube 5–6 mm long; lobes 4 equal or 5 unequal, linear, 3–5 mm long, slightly shorter than tube. Corolla pink, tube 10–13 mm long, bent and expanded proximally within calyx teeth, pubescent or glandular-pubescent; lobes of lower lip 4–7 × 2–4 mm, spreading, oval, upper lip 2–3 × 3–4 mm, obovate, emarginate.

A very distinct witchweed, stiffly erect with bright pink corollas, *S. klingii* is usually found in small populations parasitizing grasses in the wet savannas of west and central Africa. Its range extends from Senegal to southern Sudan between 4°0′N and 15°0′N with disjunct localities in Tanzania and Malawi (Fig. 13). *Striga klingii* is similar to *S. macrantha*; both species have three terminal branches just below the inflorescence. However, *S. klingii* has pink flowers whereas *S. macrantha* has white flowers and glandular hairs.

The vestiture of *S. klingii* is unique, as noted by Neumann et al. (1996) and Musselman (unpublished), with curved hairs that have very broad shield-like bases.

Selected specimens examined. CAMEROON. **Northern:** 10 km S of Ngaoundere, Adamaoua Plateau, 7°16′N, 13°35′E, *Breteler 258* (K); 7°20′N, 13°32′E, *Musselman et al. s.n.* (K); Ngaouyanga, 29 km N of Dogba, 7°20′N, 13°32′E, *Musselman* 10/16/88 (ODU). CENTRAL AFRICAN REPUBLIC. **Bamingui Bangoran:** Camp Koumbala, 8°29′N, 21°13′E, *Fay 6021* (K, MO). GHANA. **Brong Ahafo:** N of Krachi, 7°55′N, 0°1′W, *Morton 6292* (K). GUINEA. **Northern:** Youkounkoun, Sambailo, 12°40′N, 13°22′W, *Adam 13884* (MO). **Southern:** Mamou, 10°27′N, 12°2′W, *Shuell 6785* (K). GUINEA BISSAU. **Eastern:** Madina, 11°55′N, 14°5′W, *Espirito Santo 3754* (K). **Northern:** Bafata, Bambadinea, 12°12′N, 14°42′W, *Espirito Santo 3808* (K, MO); Farim, 12°35′N, 15°10′W, *Espirito Santo 3622* (K, MO); Piche, 12°25′N, 13°55′W, *Pereira 2328, 2368* (K); São Domingos, 12°30′N, 16°8′W, *Espirito Santo 3668* (K, MO). MALAWI. Kyimbila, N Lake Nyasa, *Stolz 2500* (GH). NIGER. *Barter 158* (GH). NIGERIA. **Gongola:** Vogel Peak Area, 8°22′N, 11°55′E, *Hepper 1397* (K). **Kaduna:** Zaria, Burra, 11°0′N, 9°0′E, *Harris 10* (K). **Niger:** near Mokwa, 9°18′N, 5°2′E, *Musselman & Mansfield 5533* (ODU). **North Nigeria:** *Lely 680* (K), *Roger 758* (K). SENEGAL. **Oriental:** Kedougou, 12°44′N, 12°15′W, *Adam 26881* (MO). SIERRA LEONE. **Eastern:** between Kainkordu and Sefadu, 8°34′N, 10°54′W, *Morton 2527* (K). **Northern:** Fiedwont, 8°45′N, 11°28′W, *Towa 8166* (K); Sekurela, Kabala, 9°33′N, 11°34′W, *Adam 21995* (GH, MO); Tingi Mountains, 8°56′N, 10°48′W, *Morton & Gledhill 3035* (K). SUDAN. **Equatoria:** Ibikes, 40 mi. NW of Yei, 4°17′N, 30°32′E, *Meyers 7867* (GH).

20. Striga latericea Vatke, Linnaea 43: 311. 1882. TYPE: Somalia. (?) *Anonymous s.n.* (holotype, K!).

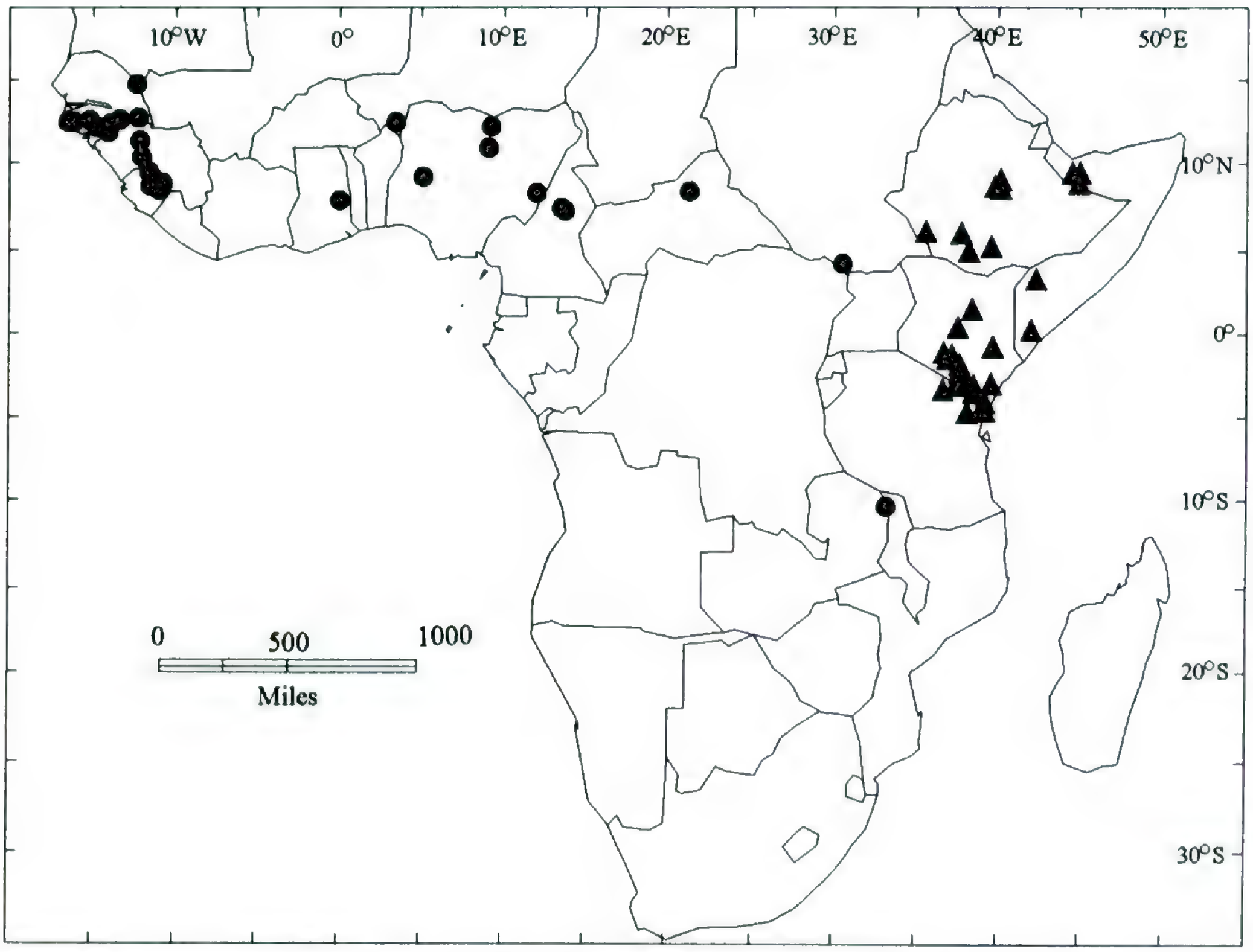


Figure 13. Distribution based on specimens of *Striga klingii* (circle) and *S. latericea* (triangle).

Rhamphicarpa stricta Engl., Abh. Preuss. Akad. Wiss. 24–39. 1894. TYPE: Tanzania. *Holst 2532* (holotype, K!).

Perennial, to 60 cm tall, stiffly erect, unbranched or with 2 to 3 branches from base, pubescent, stem square, furrowed. Leaves 20–35 × 4–6 mm, opposite, linear to lanceolate, 3-veined, shorter than internodes, margins slightly toothed. Lower bracts 20–33 × 2–6 mm, linear, often shorter than calyx, upper bracts lanceolate, shorter than calyx. Flowers opposite in racemes, raceme lax at its base, dense above middle, shorter than vegetative stem. Calyx at least 15-ribbed, 10–24 mm long; tube 10–15 mm long; lobes 5, unequal, broadly lanceolate, 5–15 mm long, longer than tube, lobes of lower calyces about 2 cm long, 3-veined. Corolla salmon-pink, tube 22–30 mm long, bent and expanded distally above calyx, densely glandular-pubescent; lobes of lower lip 10–15(–20) × 7–9 mm, obovate, upper lip 7–13 × 13–14 mm, emarginate.

Unlike *S. forbesii,* which is widely distributed in Africa, *S. latericea* (its closest putative relative) is confined to East Africa. It is found in swampy areas in Somalia, Ethiopia, Kenya, and Tanzania between 9°37′N and 4°40′S (Fig. 13) where it is sympatric with *S. forbesii.* We consider these two species distinct (also see comment under *S. forbesii*). Parker (1988) has shown that *S. latericea* is a perennial that produces aerial shoots from adventitious buds developing from the roots, unique in the genus (see also Raynal-Roques, 1993, for a discussion of roots in this species). The aerial parts also have a perennial habit with new shoots arising from the lower nodes after much of the stem matures and dies. *Striga latericea* is a pest of sugar cane in Somalia and Ethiopia (Parker & Riches, 1993).

Selected specimens examined. ETHIOPIA. **Kefa:** Maji, 6°8′N, 35°37′E, *Kaesssner 1474* (BM). **Ogaden Harerge:** Awash National Park, Metahara Plain, 8°59′N, 39°58′E, *Gilbert 1122* (K); Harar Sugar Estate, Awash Valley, 9°4′N, 40°9′E, *Parker 4034* (K, ODU). **Shewa:** Yerer and Kereya, 8°54′N, 39°59′E, *Puff et al. 8209* (K). **Sidamo:** 15 km NE Yavello, 4°59′N, 38°13′E, *Gilbert & Jefford 4594* (K); 20 km S of Negele, 5°15′N, 39°36′E, *Tadesse & Vollesen 3073* (K); Sidamo, 6°2′N, 37°46′E, *Putt & Kelbessa 921220* (K). KENYA. Between Samburu and Mackinnin, *Drummond & Homsley 4086* (K). **Coast:** between Makindu and Mtito Andei, 2°25′S, 37°57′E, *Hale 105* (K); Kilifi, near Ganze, 3°0′S, 39°35′E, *Robertson 4121* (K); Kwale, Kaya Bombo, 4°15′S, 39°10′E, *Luke 3489* (K); Mwatati-Voi, 3°10′S, 38°32′E, *Napier 957* (K); Mwatati-Voi, 3°25′S, 38°32′E, *Joanna 8928* (K); Saka–Garissa Road, 0°45′S, 39°40′E, *Faden & R.B. 74/744* (K); Vanga, Tanzania-Kenya border, 4°35′S, 39°12′E, *Smith* 10/92 (K); Diwanya Dakebuko, *Parker GM/522/H* (K). **Eastern:** Machakos, Sultan Hamud, Subalata, 2°0′S, 37°40′E, *Verdcourt 3690* (K); Mjiya Chumvi Isiolo, 0°24′N, 37°33′E, *Adamson 615* (K); Sultan Hamud, 2°8′S,

37°40′E, *Kaessner 645* (BM, K); Mwachi, 3 mi. S of Mazeras, *Drummond & Homsley 4247* (K); Mwea Irrigation Scheme, *Parker 2984* (ODU). **Nairobi:** Embakasi, near airport, 1°17′S, 36°48′E, *Napper & Faden 1839* (K); 20 km E of Nairobi, 1°17′S, 37°4′E, *Bogdan 3270A* (K); outside Nairobi at Mombasa Road, 1°19′S, 36°49′E, *Hensen 854* (K); near Magi Ghumor, *Kaessner 474* (K). **Northern:** *Jex-Blake 6880* (K). **Rift Valley:** Emali and Sultan Hamud, 2°50′S, 37°57′E, *Bally 8601* (K); Emali Station, 2°5′S, 37°27′E, *Bally 8176* (K); Majiya Chumki, 0°42′N, 37°33′E, *Adanson 615* (K, S). SOMALIA. Goan Libah, *Bally 10025* (K). **Jobba Hoose:** Juba Sugar Project, 0°15′N, 42°0′E, *Terry 3384* (K, ODU). **Woqooyi Gelbeed:** 80 km E of Hargesia, 9°37′N, 44°47′E, *Lavranos 7394* (K); Gombale Plains, 40 mi. E of Hargesia, 9°37′N, 44°38′E, *Glover & Gilliland 1231* (BM, K). TANZANIA. **Arusha:** Lekuruki Area, 3°20′S, 36°40′E, *Richards 24897, 26863* (K). **Kilimanjaro:** Mkomazi, 4°40′S, 38°7′E, *Harris 9* (K).

21. Striga lepidagathidis A. Raynal, Bull. Mus. Natl. Hist. Nat., B, Adansonia 224–225. 1987. TYPE: Guinea. Mamou, *Jacques-Felix 775* (holotype, P!).

Plant perennial, up to 12 cm tall, succulent, tufted, branched from base, glandular-pubescent, stem whitish or purple, square, furrowed. Leaves 8 × 2 mm, lanceolate, reduced, appressed, longer than internodes, margin entire, veins obscure. Bracts similar, 6 × 2 mm, lanceolate, slightly longer than calyx. Flowers imbricate in dense spikes, spike longer than vegetative stem. Calyx 5-ribbed, 6 mm long; tube 4 mm long; lobes 5, unequal, subulate, 1.5–2 mm long, about half tube length. Corolla purple, pink, or white, tube 10–12 mm long, bent and expanded proximally within calyx teeth, densely pubescent; lobes of lower lip 4–5 × 2 mm, spreading, lanceolate, longitudinally rolled in when dry, tips acute, upper lip 2–3 × 2–3 mm, emarginate, as long as wide.

Striga lepidagathidis grows in Senegal, Guinea, and Guinea Bissau (Raynal-Roques, 1987) between 13°N and 10°23′N (Fig. 8). It is parasitic on *Lepidagathis* (Acanthaceae) species and other dicotyledon plants (Raynal-Roques, 1987). *Striga lepidagathidis* resembles *S. gesnerioides* and *S. gastonii* in their overall features and is difficult to distinguish from them. (See comments under *S. gastonii*.)

Selected specimens examined. GUINEA. Touba-Kande, *Roberty 16608* (K). **Western:** Mamou (type), 10°23′N, 12°5′W, *Jacques-Felix 1241* (P). GUINEA BISSAU. **Eastern:** Boe-Madina, 11°45′N, 14°13′W, *Espirito Santo 3199* (K), *Pereira 2984* (K).

22. Striga lutea Lour., Fl. Cochinc: 22. 1790. TYPE: China. Canton, *Loureiro s.n.* (holotype, P!; holotype, K photo!).

Striga lutea var. *bicolor* Kuntze, Rev. Gen. Pl. 3, 2: 240. 1898. TYPE: Mozambique. *Kuntze 7494* (holotype, K!).

Annual, 7–40 cm tall, slender, unbranched or with 2- to 3-branches from middle, densely hispid, stem obtusely square. Leaves reduced, 3–7 × 1 mm, alternate, narrowly lanceolate, appressed, shorter than internodes, margin entire, veins obscure. All bracts similar, 1–4 × 0.5–1 mm, lanceolate, shorter than calyx. Lower surface of leaf and bract with two rows of hispid hairs along margins and midrib, upper surface hispid only along margins. Flowers sparse, alternate in lax racemes, raceme shorter than vegetative stem. Calyx 10-ribbed, 4–6 mm long; tube 3–4 mm long; lobes 5, equal, subulate, 1–2 mm long, about half tube length. Corolla yellow or rarely red, tube 10–11 mm long, bent and expanded distally above calyx, sparsely to densely glandular-pubescent; lobes of lower lip 2–3(–5) × 1–2 mm, obovate or oval, upper lip 1–2 × 2–3 mm, emarginate.

Striga lutea occurs between 15°N and 15°S (Fig. 14). The plants are easily overlooked in the field because they are small, possess a slender unbranched stem, and have reduced leaves. We do not know the relationship between this species and the Indian *S. lutea*. See notes under *S. asiatica* and *S. hirsuta*.

Selected specimens examined. BURKINA FASO. **Haut Bassins:** 5 km S of Banfora, 10°37′N, 4°43′W, *Safa & Musselman* 10/13/87 (ODU); Niangokolo Agronomic Research Station, 10°16′N, 4°54′W, *Safa & Musselman* 10/25/87 (ODU). CAMEROON. **Western:** Bangwa, 15 km NW of Bangante, 5°12′N, 10°36′E, *de Wilde & de Wilde-Duyfjes 2347* (K); Bawwda, Buru, 5°10′N, 10°30′E, *Howaiduud 1599* (K). CENTRAL AFRICAN REPUBLIC. **Ouaka:** 5 km E of Bambari, 5°41′N, 20°41′E, *Fay 1896B* (K). CONGO. **Haut-Mbomou:** Route Gwwane-Zemio, 4°55′N, 25°30′E, *Bousique 168* (K). DEMOCRATIC REPUBLIC OF CONGO. **Shaba:** Albertville, Lake Tanganyika, 5°56′S, 29°12′E, *Linder 1927* (GH). KENYA. **Coast:** Kwale, Shimba Hills, Giriama Point, 4°15′S, 39°10′E, *Magogo & Estes 1152, 1153* (K). **Eastern:** Embu, 0°32′S, 37°28′E, *Graham 2154* (K); Mahadra, *Graham 1701* (K). **Rift Valley:** Chyulu Hills, 2°32′S, 37°47′E, *Bally 8068* (K), *Gilbert* 6215 (K). MALI. **Koulikoro:** Dialakoro, 32 km S of Bamako, 12°20′N, 7°56′W, *Musselman & Mohamed* 10/4/88 (ODU); just N of Bamako, 12°40′N, 8°0′W, *Parker 4001* (ODU); Kamesoba, between Bamako-Siby, 12°30′N, 8°16′W, *Musselman & Mohamed* 10/2/88 (ODU); Zantiebougou, S of Baguineda, 12°45′N, 7°46′W, *Musselman & Mohamed* 10/5/88 (ODU). NIGERIA. **Niger:** Bida/Mokwa Junction, National Grain Corporation, 9°20′N, 5°40′E, *Parkinson* 9/18/85 (ODU), *Mansfield* 9/4/78 (ODU). SIERRA LEONE. **Northern:** near Mapaki, 9°40′N, 11°50′W, *Sighton 1298* (BM). **Western:** Waterloo, 8°25′N, 13°5′W, *Sighton 2042* (BM). TANZANIA. **Tanga:** Pombwe, Madanga, 4°50′S, 39°0′E, *Tanner 2954* (K). UGANDA. **Central:** Bugoye, 0°34′N, 33°45′E, *Dummer 2629* (US), *Curtis 4* (GH).

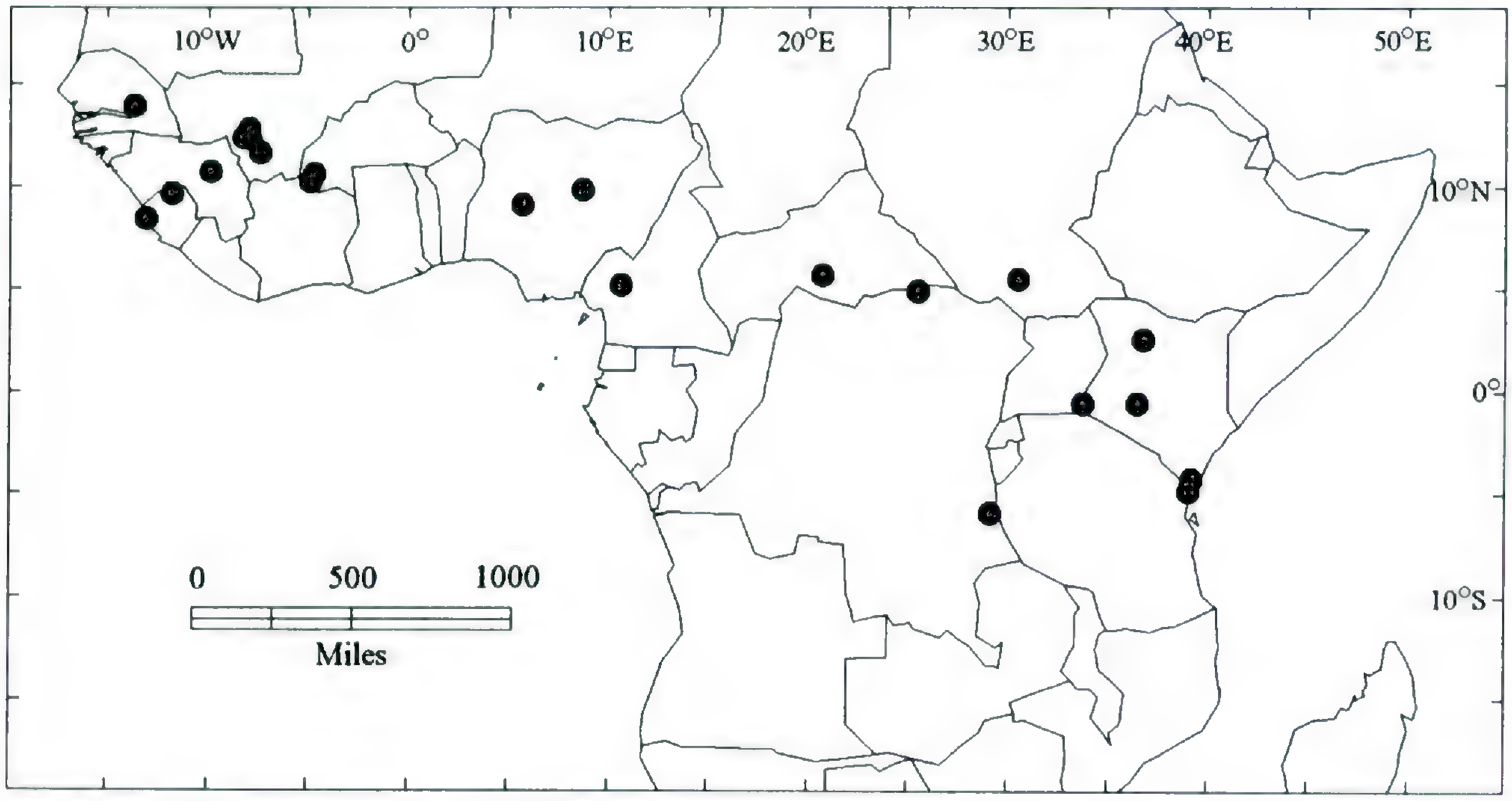


Figure 14. Distribution based on specimens of *Striga lutea* (circle).

23. Striga macrantha (Benth.) Benth., in DC. Prodr. 10: 503. 1846. *Buchnera macrantha* Benth., Companion Bot. Mag. 1: 366. 1836. TYPE: Sierra Leone. *Afzelius s.n.* (holotype, K!).

Buchnera buettneri Engl., Chev. Bot. 475. 1894. TYPE: Angola. *Welwitsch 5907* (holotype, K!).

Plant annual, up to 2.5 m tall, coarse, stiffly erect, often 3-branched just below inflorescence, covered with dense, hispid, glandular hairs, stem square, furrowed. Leaves 50–120(–160) × 4–15(–20) mm, opposite, narrowly elliptic, 3-veined, coarsely toothed, longer than internodes. All bracts similar, 10–20 × 2–5 mm, lanceolate, recurved, as long as or longer than calyx. Flowers imbricate forming very compact racemes, raceme much shorter than vegetative stem. Calyx 10-ribbed, 7–10 mm long; tube 4–7 mm long; lobes 5, unequal, lanceolate, 2–4 mm long, about half tube length. Corolla white, tube 17–24 mm long, bent and expanded distally above calyx, densely pubescent; lobes of lower lip 7–10 × 4–8 mm, obovate, spreading, upper lip 3–7 × 4–10 mm, emarginate.

One of the tallest of all witchweeds, *S. macrantha* is found in the grassland savannas in West and Central Africa between 13°55′N and 3°51′N (Fig. 12) and also from a few localities in Angola and Zambia. Its range overlaps with *S. klingii*, but *S. macrantha* has a more southerly distribution in West Africa (Figs. 12, 13). *Striga macrantha* has unique glandular trichomes composed of a 10–16-celled glandular head (Neumann et al., 1996). It resembles *S. klingii* and *S. angolensis*. See notes under *S. klingii*.

Selected specimens examined. ANGOLA. **Luanda:** Cazengo, 8°47′S, 13°15′E, *Gossweller 558* (BM). **Lunda North:** Luiballa, 9°10′S, 19°15′E, *Mouteiro 6143* (K). CAMEROON. **Center South:** Mountain Febem, 3 km NW Yaounde, 3°51′N, 11°35′E, *Breteler 2565* (K). **Littoral:** Douala to Mountain Bambouto, 4°2′N, 9°48′E, *Meurillon CNAD577* (K). **Northern:** 29 km N of Dogba at Ngaouyanga, 7°20′N, 13°32′E, *Musselman et al.* 10/16/88 (ODU). CENTRAL AFRICAN REPUBLIC. **Bamingui-Bangoran:** 8 km S of Koumbala, 8°26′N, 21°15′E, *Fay 4083* (K). GAMBIA. *Brookes 4* (K), *Lester 34* (K). GHANA. **Brong-Ahafo:** Techiman, 7°38′N, 1°58′W, *Adams & Akpabla 4509* (K). **Northern:** between River Oti and Atafie, 10°0′N, 0°25′E, *Morton 94* (K). **Volta:** Ho, 6°35′N, 0°30′E, *Glumptre 25* (K). GUINEA. **Northwest:** Youkounkoun, Sambailo, 12°40′N, 13°22′W, *Adam 13885* (MO). GUINEA BISSAU. **Central:** Bafata, Sallinho, 12°12′N, 14°42′W, *Espirito Santo 3636* (K). **Northern:** Farim, 12°35′N, 15°10′W, *Toul Botanica Exped. 1699* (K). **Western:** Tussubi, 12°0′N, 15°35′W, *Toul Botanical Exped. 1464* (K). LIBERIA. **Lofa:** Inpaka, near Kolahun Summit, 8°12′N, 10°4′W, *Bequaert 35* (GH). **Sinoe:** Greenville, 5°30′N, 8°50′W, *Adam 24394* (MO), *Adam 29806* (US). MALI. **Koulikoro:** Bamako, Manduingues, 12°40′N, 7°59′W, *Adam 11339* (MO). NIGER. *Parker 985* (GH). NIGERIA. **Bauchi:** Fuel Plantation, SE of Gombe, 10°12′N, 11°15′E, *Lowe 1484* (K). **Benue:** 2–3 mi. SE Oturkpo, 7°15′N, 8°10′E, *Anonymous 2143* (BM). **Gongola:** Adamawa, Dakemi, 8°25′N, 11°50′E, *Hepper 1392* (K). **Kaduna:** Zaria, near dam for Institution for Agriculture Research, 10°58′N, 7°58′E, *Gbile & Daramola 245* (K). **Kwara:** 10 km W of Isanlu-Makutu, 8°15′N, 5°45′E, *Lowe 4463* (K). **Lagos:** W of Lagos, 6°28′N, 3°30′E, *Rowland 1893* (K). **Niger:** 45 mi. N of Bida, 9°29′N, 5°58′E, *Harris 8* (K). **Northern:** *Dalziel 169, 911* (K). **Ogun:** Abeokuta, 7°6′N, 3°25′E, *Baldwin 13639* (K, MO, US). **Oyo:** Oyo, University of Ife Camp, 7°58′N, 4°0′E, *Suabad 6994* (MO). **Plateau:** Jos Plateau, 9°50′N, 8°40′E, *Batten-Poole 226* (K). **Southern:** southern Guinea Savana, *Musselman & Mansfield 5522* (ODU). SENEGAL. **Oriental:** Kedougou, 12°44′N, 12°15′W, *Adam 20029* (MO). SIERRA LEONE. **Freetown:** rifle range, near Waterloo,

8°22′N, 13°3′W, *F.A.M. & T.H. 474* (K). **Northern:** Bisawa, above Bumbuna, 9°6′N, 11°44′W, *King 175* (K). SUDAN. **North Darfur:** Ngertete, Gabal Marra, 12°50′N, 24°30′E, *Wickens 2807* (K). CHAD. **Logone-Oriental:** Lere to Ham, at 9°50′N, 14°40′E, *Talbot 324* (BM, K). ZAMBIA. **Southern:** Kalomo, 17°0′S, 26°30′E, *Fanshawe 4689* (K).

24. Striga passargei Engl., Bot. Jahrb. 23: 515, 12 figs. M, N. 1897. TYPE: Cameroon. Jola, *Passarge 48* (holotype, K!).

Annual, to 46 cm tall, erect, unbranched or branched from middle, densely hispid, stem obtusely square. Leaves 10–45 × 1–3 mm, narrowly elliptic, opposite/subopposite, ascending, longer than internodes, margin entire, veins obscure. Lower bracts 12–60 × 1–3 mm, linear, much longer than calyx, upper bracts as long as or longer than calyx. Flowers alternate in long, lax spikes, spike longer than vegetative stem. Calyx 5-ribbed, 8–12 mm long; tube 3–8 mm long; lobes 5, equal, linear to lanceolate, 3–8 mm long, about same length as tube. Corolla cream-white, rarely pink or yellow, tube 6–8 mm long, bent proximally within calyx teeth, glandular-pubescent; lobes of lower lip 2–6 × 2–4 mm, round, upper lip 1–3 × 2–5 mm, emarginate.

Striga passargei is found in the wet areas within the Sudanian domain in Africa. Its range extends from Senegal to Sudan between 14°44′N and 6°35′N (Fig. 15). In this area it is sympatric with *S. aspera* and *S. brachycalyx*. Unlike these two species, *S. passargei* always occurs in smaller populations. South of the equator, it occurs only in Tanzania, Zambia, and Namibia (Fig. 15). There is considerable variation in populations of *S. passargei* particularly in stem height, leaf and bract size, calyx lobes relative to tubes, and corolla color.

Selected specimens examined. BURKINA FASO. **Central:** Zorgo, 69 km E of Ouagadougou, 12°24′N, 0°35′W, *Raynal 22277* (K). **Center East:** 5 km S of Ouada, 11°30′N, 0°20′W, *Safa & Musselman* 10/9/87 (ODU). **Center North:** 55 km N of Ouagadougou, 12°51′N, 1°39′W, *Safa & Musselman* 10/19/87 (ODU). CAMEROON. **Northern:** Bogo, 35 km from Maroua, 10°44′N, 14°36′E, *Bounougou 126* (K). GHANA. **Brong-Afafo:** Bambo, 8°10′N, 2°12′W, *Hall 2028* (K). GUINEA **Northern:** Jiripa, 10°55′N, 1°58′W, *Thorold 251* (BM). **Central:** Pita, 11°6′N, 12°24′W, *Adames 341* (K). MALI. **Koulikoro:** Dialakoro, 32 km S of Bamako, 12°18′N, 7°56′W, *Musselman & Mohamed* 10/4/88 (ODU). NAMIBIA. **Ovamboland:** Ondangua, 17°56′S, 16°0′E, *Kers 1399* (S). NIGERIA. **Bauchi:** Panshanu Pass, 10°5′N, 9°3′E, *Lawlor & Hall 461* (K). **Bornu:** Lake Tchad Area, 13°5′N, 13°51′E, *Parkinson A1* (K). **Gongola:** Yola, 9°10′N, 12°25′E, *Dalziel 110* (K). **Kaduna:** Panshanu, 30 mi. from Jos on Jos–Bauchi Road, 10°8′N, 8°0′E, *Lawlor & Hall 73* (K). **Ogun:** Ibadan, IITA, 7°17′N, 3°30′E, *Parkinson 1985* (ODU). **Plateau:** Jos, Naraguta Hills, SE Naraguta, 9°50′N, 8°40′E, *Lawlor & Hall 34* (K). **Sokoto:** Sokoto, 13°8′N, 5°15′E, *Dalziel 355* (K). SENEGAL. **Oriental:** Dialakoto, 13°27′N, 13°18′W, *Musselman et al. 7061, 7063* (ODU). SUDAN. **Blue Nile:** Abu Naama, Gabal Tozi, 12°54′N, 33°36′E, *Lea 58* (K, ODU), *Lea 180* (K). **Jungali:** near Baidit, 45 km N of Bor, 6°35′N, 31°36′E, *Lock 81/248* (K). **North Darfur:** Kutum, 14°10′N, 24°42′E, *Lynes 578* (BM). **North Kurdufan:** Al-Obied, 13°15′N, 30°12′E, *Pfund 86* (K). **South Kurdufan:** Kadugli, Saref Research Station, 11°5′N, 29°42′E, *Musselman 198* (K). TANZANIA. **Eastern:** 2 mi. W of Chibembe River, Luangwa Valley Game Reserve, 14°25′S, 30°30′E, *Prince 200, 401* (K). **Lindi:** Kingupira Forest, 8°30′S, 38°34′E, *Vollesen 3311* (K, UPS). **Mwanza:** Mwanza, 2°30′S, 32°58′E, *Davis 255* (K). **Rukwa:** Tumba, Rukwa Valley, 4°15′S, 33°14′E, *Siame 144* (K). **Shinyanga:** near Shinyanga, 3°47′S, 33°27′E, *Rax 292* (K). **Tabora:** Rukwa-Nzega Plain, 4°10′S, 33°12′E, *Whellar 1188* (K). TOGO. **Northern:** Dapaon, (Dapango), 10°44′N, 0°12′E, *Hakki et al. 1504* (K). ZAMBIA. **Mashonaland North:** Kariba Airport, 16°30′S, 28°35′E, *Philcox & Leppard 8677* (K).

25. Striga pinnatifida Getachew Aweke, Kew Bull. 47: 293–294. 1992. TYPE: Ethiopia. Sidamo Prov., *Friis, Gilbert, Rasmussen & Vollesen 918* (holotype, K!; isotype, ETH not seen).

Annual, to 45 cm tall, erect, unbranched or with 2 to 3 branches from middle, covered with dense, hispid hairs, stem obtusely square. Leaves 15–30 × 10 mm, alternate, pinnatifid with linear segments, shorter than internodes. Lower bracts 10–25 × 8 mm, leaf-like, longer than calyx, upper bracts shorter than calyx. Flowers shortly pedicellate, alternate, axillary, solitary, sparse. Calyx 10-ribbed, 10–12 mm long; tube 6–7 mm long; lobes 5, unequal, lanceolate, 3–5 mm long, half tube length. Corolla white, tube 20 mm long, bent and expanded distally above calyx, pubescent; lobes of lower lip 6 × 5 mm, spreading, obovate, upper lip 3 × 6 mm, emarginate.

Striga pinnatifida is known solely from the type collection. It is the only witchweed with pinnatifid leaves and solitary flowers.

26. Striga primuloides Chev., Bull. Soc. Bot. Fr. Mem. 8: 185. 1912. TYPE: Ivory Coast. Anoumaba, *Chevalier B.22.404* (holotype, P!; isotype, K!).

Perennial, 30–70 cm tall, erect, unbranched, appearing leafless, pilose, stem obtusely square. Leaves scale-like, 2–5 × 1–2 mm, lanceolate, alternate, appressed, shorter than internodes, margin entire, veins obscure. All bracts similar, 4–7 × 1–2 mm, lanceolate, shorter than calyx. Flowers sparse, alternate in lax spikes, spike much shorter

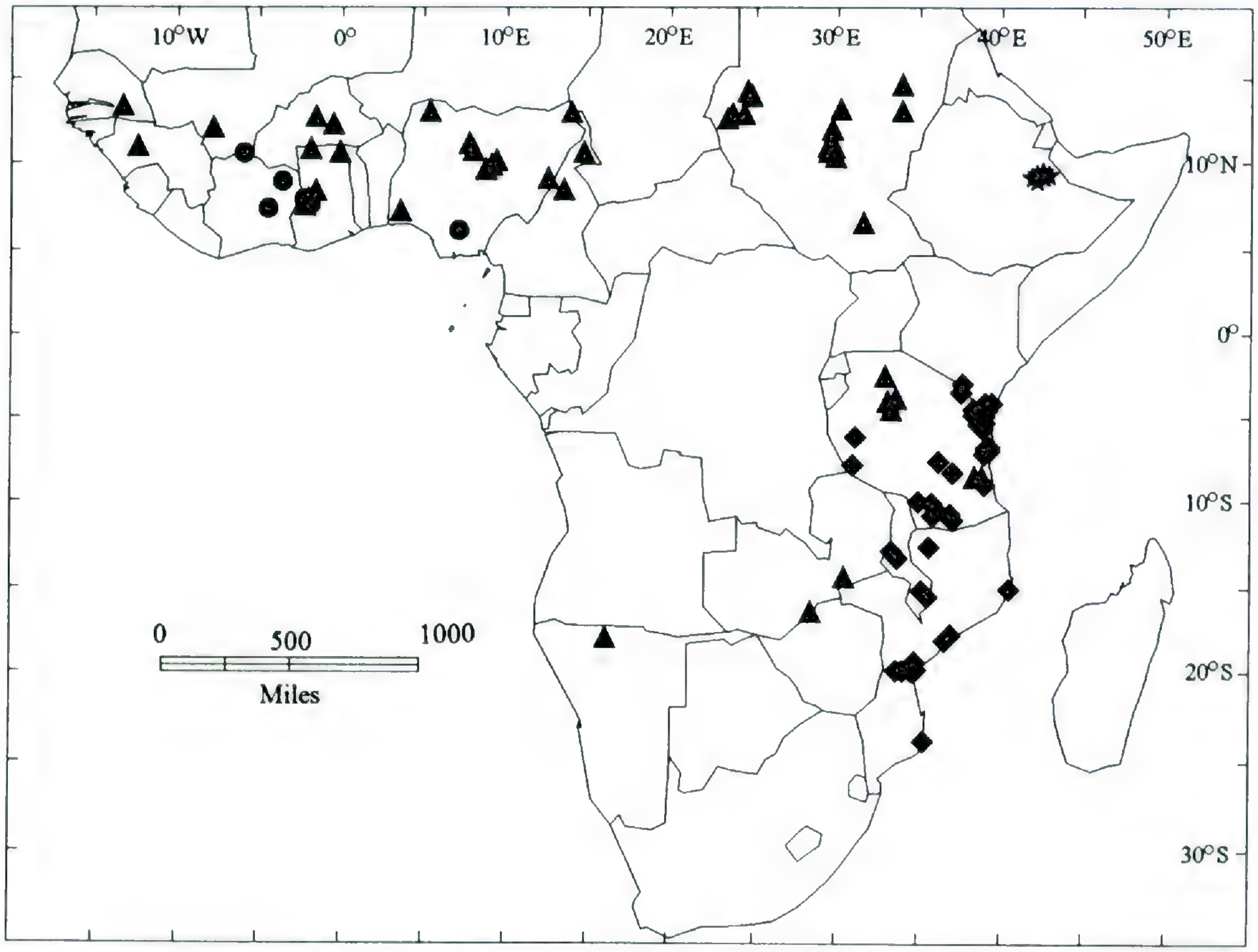


Figure 15. Distribution based on specimens of *Striga passargei* (triangle), *S. primuloides* (circle), *S. pubiflora* (diamond), and *S. yemenica* (star).

than vegetative stem. Calyx approximately 15-ribbed, 8–9 mm long; tube 5 mm long; lobes 5, unequal, lanceolate, 3–4 mm long, slightly shorter than tube. Corolla pale yellow, tube 22–30 mm long, bent and expanded distally above calyx, densely glandular-pubescent; lobes of lower lip 9–10 × 8–9 mm, round, spreading, as long as wide, upper lip 5 × 10 mm, broadly obovate, notched.

Striga primuloides is restricted to the southern parts of West Africa between 5°10′N and 10°37′N (Fig. 15). It is a rare species with only a few collections from Mali, Ivory Coast, Ghana, and southern Nigeria.

Selected specimens examined. GHANA. **Brong-Ahafo:** Bambo Ravine, 8°10′N, 2°8′W, *Hall 1998* (K), *Morton A4241* (K); Bambo, W of Wenchi, 8°15′N, 2°12′W, *Morton GC8601* (K). **Northeastern:** along road from Gawi to Seye, in Gallery Forest of Iringou River, 9°14′N, 3°41′W, *Amshoff 193* (MO). MALI. **Mopti:** Massina, Diou, 14°52′N, 5°16′W, *Laferrere 112* (K). NIGERIA. **South Nigeria:** Awkaka, *Birkett-Smith 49* (K).

27. Striga pubiflora Klotzsch., in Peters Reise Mossamb. Bot.: 227. 1861. TYPE: Mozambique. *Dayser s.n.* (holotype, B not seen).

Striga zanzibarensis Vatke, Linnaea 43: 310. 1882. TYPE: Mafia Isl. *Hildebrandt 1904* (holotype, K!).

Perennial, 35–75 cm tall, stiffly erect, unbranched or branched from base, densely pilose or hispid, stem obtusely square, furrowed. Leaves 10–20(–50) × 1–2(–4) mm, linear, opposite on the lower part of the stem, alternate above, shorter than internodes, margin entire, rarely toothed, veins obscure. Lower bracts 22–27 × 2 mm, linear, longer than calyx, upper bracts shorter than calyx. Flowers alternate in lax racemes, raceme shorter than vegetative stem. Calyx 15-ribbed, 13–21 mm long; tube 8–12 mm long; lobes 5, equal, lanceolate, 4–12 mm long, as long as or shorter than tube. Corolla white, tube 27–35 mm long, bent and expanded distally above calyx, densely pubescent; lobes of lower lip 14–20 × 10–17 mm, obovate, spreading, upper lip 9–12 × 10–20 mm, widely obovate.

Striga pubiflora is common in the seasonally flooded savannas of southeastern Africa between 3°10′S and 23°54′S (Fig. 15). Its range extends from Kenya south into Tanzania, Zanzibar, Malawi, and Mozambique. It may be related to *S. junodii* and *S. angustifolia* (Mohamed, 1994). *Striga pubiflora* and *S. angustifolia* are sympatric and also have white flowers, but the corolla of *S. pubiflora* is much larger.

Selected specimens examined. KENYA. **Coast:** Kwale,

Shimba Hills, Mombasa Road between Vanga and Tiwi turn-off, 4°25′S, 39°8′E, *Magogo & Glover 1050* (K). MALAWI. **Central:** Dowa, 25 km S of Kasungu, 13°14′S, 33°40′E, *Pawek 4112* (K). **Southern:** Zomba Mountain, Chivunde Valley Road, 15°20′S, 35°19′E, *Croix 2735* (MO). MOZAMBIQUE. 5 mi. NE of Lunga, *Moomaw 953* (K). **Inhambane:** Inhambane, 23°54′S, 35°30′E, *Anonymous s.n.* (BM). **Manica:** 25 mi. S of Muda, 19°51′S, 33°50′E, *Leach 11232* (K, MO). **Niassa:** 47 mi. W of Nova Ureixo on Mandimba Road, 12°40′S, 35°40′E, *Leach & Smith 11011* (K), *Allen 84* (K), *Stocks 55* (K). **Sofala:** 41 mi. N of Sofala, 19°50′S, 34°34′E, *Methew 257* (K). **Zambizia:** Quelimane, 17°53′S, 36°58′E, *Faulkner K118* (K). TANZANIA. **Dar es Salaam:** 20 mi. S of Dar es Salaam, between Mbwa and Kimbiji, 7°0′S, 39°18′E, *Batty 870* (K). **Iringa:** Mahenge Plateau, 7°37′S, 36°12′E, *Schlieben 2281* (K). **Lindi:** 3 km W of Nainokwe/Njinjio-Nangurukuru Road junction on road to Kilwa, 9°0′S, 39°0′E, *Magogo & Innes 373* (K). **Mafia:** Mji Mkuu, Kipumbwe, Mwera, *Tanner 2850* (K). **Morogoro:** 3 km N of Mlahi, 8°16′S, 37°5′E, *Kesollesen 2817* (K). **Pwani:** Kibaha, N of Bush, 6°46′S, 38°55′E, *Harris 786* (S). **Rukwa:** Mlimba, 7°51′S, 31°0′E, *Paget-Wilkes 700* (MO). **Ruvuma:** 5 km E of Gumbiro, 10°15′S, 35°48′E, *Milne-Redhead & Taylor 8421* (K). **Tanga:** Aboni, Mgaza 4°50′S, 39°0′E, *Tanner 89* (K). **Wani:** Kisarawe Mogo Forest Reserve, 7°3′S, 39°0′E, *Poulo 105* (K, MO). **Zanzibar:** Fessland bec Lamu, *Hildebrandt 1907* (UPS).

28. Striga yemenica Musselman & Hepper, Notes Roy. Bot. Gard. Edinburgh 45(1): 43–50. 1988. TYPE: Yemen Arab Republic. Jiblah, *Miller 546* (holotype, E!; isotype, K!).

Annual, 18–22 cm tall, erect, usually unbranched, covered with dense, retrorse, hispid hairs, stem square, furrowed. Leaves 10–22 × 1–2 mm, lanceolate, opposite, ascending, shorter than internodes, margin entire, veins obscure. Lower bracts 8–12 × 1(–2) mm, lanceolate, ciliate, longer than calyx, upper bracts shorter than calyx. Flowers opposite in lax spikes, spike as long as vegetative stem. Calyx 5-ribbed, 5–7 mm long; tube 4–5 mm long; lobes 5, unequal, (adaxial reduced to < 1 mm) deltate, 1(–2) mm long, much shorter than tube. Corolla red or mauve, bent distally above calyx, densely retrorsely pubescent; lobes of lower lip 0.5–1 × 2 mm, round, upper lip small, 0.5 × 2 mm, truncate.

In Africa, only in Ethiopia between 9°6′N, 9°38′N and 41°48′E, 42°49′E (Fig. 15). It bears some morphological similarities to *S. hermonthica* and *S. gracillima.* In these three species, the calyx lobes are short and deltate with the adaxial lobe reduced to less than 1 mm in length; corollas are pink or mauve with simple hairs and never glandular. *Striga yemenica,* on the other hand, has retrorse hairs in contrast to the antrorse hairs of *S. hermonthica* and *S. gracillima.*

Selected specimens examined. ETHIOPIA. 31 km from Alemaya on road to Grawa and Bedeno, *Gilbert 4072* (K). **Ogaden Harerge:** 4 km W of China Hasen, 31 km NW of Jijiga, 9°26′N, 42°42′E, *Boulos 10250* (K); Gures, 18 km NW of Jijiga, 9°20′N, 42°42′E, *Boulos 10509* (K); Harrar, 9°18′N, 42°8′E, *Ellis 272* (K); Mountain Hakim, 9°17′N, 42°6′E, *de Wilde 7223* (K); Mountain Hakim, 9°16′N, 42°7′E, *Gilbert & S.B. 1408* (K); 1 km W of Abu Yonis, 30 km NW Jijiga, 9°24′N, 42°45′E, *Boulos 10316* (K, MO).

Excluded Species

Striga baumannii has tuberous roots and a ligneous calyx, features not found in any other *Striga* species. The foliage is also unique with two large leaves formed during the growing season. In *Striga* the lowermost leaves are reduced to small scales. We consider *S. baumannii* distinct from any witchweed and therefore do not include it in the genus. It is thought to represent a new genus (Mohamed, unpublished).

Striga fulgens (= *Buchnera fulgens* Engl.) was named by Hepper (1984). However, Raynal-Roques (1993) presented evidence that it is a member of the Verbenaceae, likely in the genus *Chascanum* E. Mey. It has free lobes of the upper corolla lip as well as a glabrous calyx with characteristic white bands between the ribs and short calyx lobes (1–2 mm). Accordingly, we exclude it from *Striga.*

Literature Cited

Agbobli, C. A. 1991. Effect of nitrogen rates on *Striga asiatica* emergence in maize culture in Togo. Pp. 28–30 *in* J. K. Ransom, L. J. Musselman, A. D. Worsham & C. Parker (editors), Proceedings of the Fifth International Symposium on Parasitic Weeds. CIMMYT, Nairobi.

Aigbokhan, E. I., D. K. Berner & L. J. Musselman. 1998. Reproductive ability of hybrids of *S. aspera* and *S. hermonthica.* Phytopathology 88: 563–567.

———, ———, ——— & H. D. Mignouna. 2000. Evaluation of variability in *Striga aspera, Striga hermonthica* and their hybrids using morphological characters and random amplified polymorphic DNA markers. Weed Res. 40: 375–386.

Anonymous. 1996. MapInfo Professional, 2nd ed. MapInfo, Troy, New York.

Berner, D. K., K. F. Cardwell & B. O. Faturoti. 1992. *Striga hermonthica* distribution mechanisms and their implications in control. Phytopathology 82: 1164.

Bharathalakshmi, C. R. Werth & L. J. Musselman. 1990. A study of genetic diversity among host-specific populations of the witchweed *Striga hermonthica* (Scrophulariaceae) in Africa. Pl. Syst. Evol. 172: 1–12.

Boeshore, I. 1920. The morphological continuity of Scrophulariaceae and Orobanchaceae. Contr. Bot. Lab. Morris Arbor. Univ. Pennsylvania 5: 139–177.

Cubero, J. I. 1996. Cytogenetics in Orobanchaceae: A review. Pp. 75–96 *in* M. T. Moreno & J. I. Cubero (editors), Advances in Parasitic Plant Research. Junta de Andalucía, Córdoba.

DeLeonardis, M. A. 1986. The Pollen Exostructure of

Striga (Scrophulariaceae). M.S. Thesis, Old Dominion University, Norfolk.

Engler, A. 1897. Scrophulariaceae africanae. II. Bot. Jahrb. 23: 497–517.

Geiger, U., J. Kroschel & J. Sauerborn. 1996. *Striga asiatica*, a problem in the middle west of Madagascar. Pp. 479–486 *in* M. T. Moreno & J. I. Cubero (editors), Advances in Parasitic Plant Research. Junta de Andalucía, Córdoba.

Hepper, F. N. 1960. The *Striga bilabiata* (Thunb.) Kuntze complex in Africa. Kew Bull. 14: 411–416.

———. 1974. Parasitic witchweed: *Striga asiatica* versus *S. lutea* (Scrophulariaceae). Rhodora 76: 45–47.

———. 1984. The species of *Striga* in East Africa. Pp. 262–265 *in* C. Parker, L. J. Musselman, R. M. Polhill & A. K. Wilson (editors), Proceedings of the Third International Symposium on Parasitic Weeds. ICARDA/ International Parasitic Seed Plants Research Group, Aleppo.

———. 1986. Proposal to reject the name *Buchnera euphrasioides* Vahl (*Striga euphrasioides* (Vahl) Benth.) (Scrophulariaceae). Taxon: 35: 390–391.

Kenfack, D., L. J. Musselman & H. J. Hovers. 1996. Hosts of eight *Striga* species (Scrophulariaceae) in Cameroon. Pp. 465–470 *in* M. T. Moreno & J. I. Cubero (editors), Advances in Parasitic Plant Research. Junta de Andalucía, Córdoba.

Knepper, D. A., A. T. Obilana & L. J. Musselman. 1991. Morphology of *Striga forbesii* and preliminary screening for resistance in sorghum. Pp. 241–246 *in* J. K. Ransom, L. J. Musselman, A. D. Worsham & C. Parker (editors), Proceedings of the Fifth International Symposium on Parasitic Weeds. CIMMYT, Nairobi.

Maass, E. 1999. A Comparative Study on the Germination Requirements of Some Economically Important *Striga* Species. Ph.D. Dissertation, University of Stellenbosch.

Minkin, J. P. & W. H. Eshbaugh. 1989. Pollen morphology of the Orobanchaceae and rhinanthoid Scrophulariaceae. Grana 28: 1–18.

Mohamed, K. I. 1994. Biosystematics and Diversification in the Genus *Striga* Lour. (Scrophulariaceae) in Africa. Ph.D. Dissertation, Old Dominion University, Norfolk.

Musselman, L. J. & E. S. Ayensu. 1984. Taxonomy and biosystematics of *Striga*. Pp. 37–45 *in* E. S. Aysensu, H. Doggett, R. D. Keynes, J. Marton Le-Fevre, L. J. Musselman, C. Parker & A. Pickering (editors), Striga Biology and Control. International Council of Scientific Unions Press, Paris.

——— & F. N. Hepper. 1986. The witchweeds (*Striga*, Scrophulariaceae) of the Sudan Republic. Kew Bull. 41: 205–221.

——— & W. F. Mann, Jr. 1976. A survey of surface characteristics of seeds of Scrophulariaceae and Orobanchaceae using scanning electron microscopy. Phytomorphology 26: 370–378.

——— & C. Parker. 1981. Surface features of *Striga* seeds (Scrophulariaceae). Adansonia, series 2 20: 431–437.

———, Bharathalakshmi, S. B. Safa, D. A. Knepper, K. I. Mohamed & C. L. White. 1991. Recent research on the biology of *Striga asiatica*, *Striga gesnerioides* and *Striga hermonthica*. Pp. 31–41 *in* S. K. Kim (editor), Combating *Striga* in Africa. Proceedings, International Workshops Organized by IITA, ICRISAT and IDRC. IITA, Ibadan.

Neumann, U., J. Paré, A. Raynal-Roques, G. Sallé & H. Weber. 1996. Characteristic trichomes observed in some African parasitic Scrophulariaceae. Pp. 264–272 *in* M. T. Moreno & J. I. Cubero (editors), Advances in Parasitic Plant Research. Junta de Andalucía, Córdoba.

Nickrent, D. L., R. J. Duff, A. E. Colwell, A. D. Wolfe, N. D. Young, K. E. Steiner & C. W. dePamphilis. 1998. Molecular phylogenetic and evolutionary studies of parasitic plants. Pp. 211–241 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II. DNA Sequencing. Kulwer Academic Publishers, Boston.

Olivier, A., J. C. Glaszmann, C. Lanaud & G. D. Leroux. 1998. Population structure, genetic diversity, and host specificity of the parasitic weed *S. hermonthica* (Scrophulariaceae) in Sahel. Pl. Syst. Evol. 209: 33–45.

Olmstead, R. G., C. W. dePamphilis, A. D. Wolfe, N. D. Young, W. J. Elisons & P. A. Reeves. Disintegration of the Scrophulariaceae. Amer. J. Bot. (in press).

Parker, C. 1988. Parasitic plants in Ethiopia. Walia 11: 21–27.

——— & C. R. Riches. 1993. Parasitic Weeds of the World. CABI, Oxford.

Press, M. C. & J. D. Graves (editors). 1995. Parasitic Flowering Plants. Chapman and Hall, London.

Ralston, D. M., C. R. Riches & L. J. Musselman. 1987. Morphology and hosts of three *Striga* species (Scrophulariaceae) in Botswana. Bull. Mus. Natl. Hist. Nat., B, Adansonia 2: 195–215.

Ramaiah, K. V., C. Parker, M. J. Vasudeva Rao & L. J. Musselman. 1983. *Striga* Identification and Control Handbook. ICRISAT Information Bulletin #15, Patancheru, India.

Raynal-Roques, A. 1969. Deux nouveaux *Striga* africains (Scrophulariaceae). Bull. Jard. Bot. Belg. 39: 373–382.

———. 1987. The genus *Striga* (Scrophulariaceae) in western and central Africa—A survey. Pp. 675–689 *in* H. C. Weber & W. Forstreuter (editors), Proceedings of the Fourth International Symposium on Parasitic Flowering Plants. ISPPEP, Marburg.

———. 1991. Diversification in the genus *Striga*. Pp. 251–261 *in* J. K. Ransom, L. J. Musselman, A. D. Worsham & C. Parker (editors), Proceedings of the Fifth International Symposium on Parasitic Weeds. CIMMYT, Nairobi.

———. 1993. Contribution à la connaissance de la biologie des *Striga* (Scrophulariaceae): Types biologiques et phénologie. Bull. Mus. Natl. Hist. Nat., B, Adansonia 3–21.

Saldanha, C. J. 1963. The genus *Striga* Lour. in western India. Bull. Bot. Surv. India 5: 67–70.

Sand, P. F., R. E. Eplee & R. G. Westbrooks (Editors). 1990. Witchweed Research and Control in the United States of America. Weed Science Society of America, Champaign.

Sauerborn, J. 1991. Parasitic Flowering Plants. Ecology and Management. Verlag Josef Margraf, Weikersheim.

Thalouarn, P., M. C. Arnaud, C. Theodet & L. Rey. 1991. Cytological, biochemical and genetic aspects of carbon fixation in *Striga hermonthica* and *Striga gesnerioides*. Pp. 51–57 *in* J. K. Ransom, L. J. Musselman, A. D. Worsham & C. Parker (editors), Proceedings of the Fifth International Symposium on Parasitic Weeds. CIMMYT, Nairobi.

Werth, C. R., J. L. Riopel & N. W. Gillespie. 1984. Genetic uniformity in an introduced population of witchweed (*Striga asiatica*) in the United States. Weed Sci. 32: 645–648.

Wettstein, R. von. 1891. Scrophulariaceae. *In* A. Engler & K. Prantl (editors), Die Natürlichen Pflanzenfamilien IV (3b): 39–107.

Wild, H. 1948. A suggestion for the control of tobacco witchweed [*Striga gesnerioides* (Willd.) Vatke] by leguminous trap crops. Rhodesia Agric. J. 45: 208–215.

Young, N. D., K. E. Steiner & C. W. dePamphilis. 1999. The evolution of parasitism in Scrophulariaceae/Orobanchaceae: Plastid gene sequences refute an evolutionary transition series. Ann. Missouri Bot. Gard. 86: 876–893.

List of Species

1. *Striga aequinoctialis* A. Chev. ex Hutch. & Dalz.
2. *Striga angolensis* Mohamed & Musselman
3. *Striga angustifolia* (Don) Saldanha
4. *Striga asiatica* (L.) Kuntze
5. *Striga aspera* (Willd.) Benth.
6. *Striga bilabiata* (Thunb.) Kuntze
   - 6a. *Striga bilabiatia* subsp. *barteri* (Engl.) Hepper
   - 6b. *Striga bilabiata* Kuntze subsp. *bilabiata*
   - 6c. *Striga bilabiata* subsp. *jaegeri* Hepper
   - 6d. *Striga bilabiata* subsp. *ledermannii* (Pilger) Hepper
   - 6e. *Striga bilabiata* subsp. *linearifolia* (Schum. & Thonn.) Mohamed
   - 6f. *Striga bilabiata* subsp. *rowlandii* (Engl.) Hepper
7. *Striga brachycalyx* Skan
8. *Striga chrysantha* A. Raynal
9. *Striga dalzielii* Hutch.
10. *Striga elegans* Benth.
11. *Striga forbesii* Benth.
12. *Striga gastonii* A. Raynal
13. *Striga gesnerioides* (Willd.) Vatke
14. *Striga gracillima* H. Melchior
15. *Striga hallaei* A. Raynal
16. *Striga hermonthica* (Del.) Benth.
17. *Striga hirsuta* Benth.
18. *Striga junodii* Schinz.
19. *Striga klingii* (Engl.) Skan
20. *Striga latericea* Vatke
21. *Striga lepidagathidis* A. Raynal
22. *Striga lutea* Lour.
23. *Striga macrantha* (Benth.) Benth.
24. *Striga passargei* Engl.
25. *Striga pinnatifida* Getachew Aweke
26. *Striga primuloides* Chev.
27. *Striga pubiflora* Klotzsch.
28. *Striga yemenica* Musselman & Hepper

Index to Exsiccatae

Specimens examined are listed alphabetically by collector; followed by collection numbers (or dates of collections for specimens with no numbers), herbarium, and the species number corresponding to the number in the List of Species.

Acocks, J. P. 1481 (K) (6b), 1491 (K) (10), 2012 (K) (13); Adam, J. G. 5111 (MO) (1), 11248 (MO) (23), 11339 (MO) (23), 13724 (MO) (6f), 13884 (MO) (19), 13885 (MO) (23), 14200 (MO) (6f), 14812 (MO) (6f), 14861 (MO) (6c), 17917 (MO) (6a), 17934 (MO) (6a), 20029 (MO) (23), 21457 (K, MO, UPS) (1), 21957 (MO) (23), 21995 (GH, MO) (19), 24039 (MO) (1), 24386 (MO) (1), 24394 (MO) (23), 24653 (MO) (1), 25117 (MO) (23), 25550 (K, MO) (1), 25592 (K) (1), 26054 (MO) (1), 26531 (MO) (1), 26881 (MO) (19), 26891 (MO) (6f), 26896 (MO) (6a), 26897 (MO) (6f), 26898 (MO) (6f), 27125 (MO) (6e), 27478 (MO) (1), 27661 (MO) (6f), 29806 (US) (23); Adames, P. 146 (K) (6f), 193 (K) (23), 224 (K) (6f), 284 (K) (1), 341 (K) (24); Adams, C. D. 3548 (K) (6e), 4152 (K) (6e), 4419 (K) (16), 4472 (K) (23), 4693 (K) (7); Adams, C. D. & G. K. Akpabla 4107 (K) (16), 4272 (K) (5), 4509 (K) (23); Adanson, G. 615 (K, S) (20); Adamson, J. 527 (K) (4), 615 (K) (20), 3562 (K) (13); Agnew, A. 8700 (MO) (6e); Ajayi, M. O. 19271 (K) (23); Ake, L. 8755 (K) (6e); Akestrid 2527 (UPS) (6e); Akpabla, G. K. 36 (K) (13); Alcool, M. 963 (K) (16), 4116 (K) (6a), 4142 (K) (11); Allen, C. E. 124 (K) (13); Allen, E. F. 84 (K) (27); Allen, J. 10470 (K) (13); Amshoff, G. J. 193 (MO) (26), 225 (MO) (6e); Anderson, B. 846 (K) (4), 885 (K) (11), 1364 (K) (11); Andrews, F. W. 1018 (K) (17); Andrews, J. W. 6 (K) (5); Angus, A. 1066 (K) (6b); Ankole, E. & W. J. Eggeling 610 (K) (6e); Ankrah, J. O. 20183 (K) (6e); Anonymous 189 (K) (13), 782 (K) (13), 837 (K) (13), 1036 (K) (16), 1126 (K) (5), 1651 (K) (6e), 1784 (K) (18), 1829 (K) (6e), 1842 (GH, US) (17), 1858 (K) (11), 2069 (GH) (10), 2143 (BM) (23), 2159 (K) (4), 2180 (K) (4), 2381 (K) (16), 2482 (GH) (10), 3063 (K) (11), 3384 (K) (11), 4863 (K) (6b), 5749 (GH) (4), 8347 (K) (4), 9199 (US) (16), 9200 (K) (13), 9783 (K) (1), 11762 (K) (11), 12092 (MO, S, UPS) (18), 12092 (BM, K, US) (27), s.n. (BM) (27), s/ss (K) (16); Appelsbosch 1889 (UPS) (4); Archbold, A. E. 694 (K) (4), 732 (K) (4); Archbold, M. E. 2776 (K) (13); Aschemeier, C. R. 11 (US) (17); Ash, J. W. 347 (K) (13), 518 (K) (17), 557 (K) (13), 1089 (K) (13), 3648 (K) (16); Ash, M. J. 544 (K) (11), 2259 (K) (11); Astle, W. L. 1023 (K) (6b), 1556 (K) (11), 1571 (K) (10), 4471 (K) (11), 4570 (K) (11), 5507 (K) (4); Audru, 1245 (P) (12); Audry, P. B2 (K) (16); Awaikweki, et al. 148 (K) (11); Awbeya, 1430 (MO) (10); Aylmar, G. 27/50 (K) (11), 27/51 (K) (13e), 236 (K) (13).

Baikie, W. B. 1865 (GH) (16); Baitha, R. 36 (K) (16); Bakshi, T. S. 146 (K) (6f); Baldwin, J. T. 5988 (US) (17), 13639 (K, MO, US) (23), s.n. (K) (4); Ballenetto 277 (K) (13); Balls, E. K. 3022 (BM, K) (13); Bally, M. 283 (K) (17); Bally, P. R. O. 4596 (K) (13), 7329 (K) (13), 8064 (K) (6e), 8068 (K) (22), 8072 (K) (11), 8176 (K) (20), 8601 (K) (20), 9675 (K) (13), 10025 (K) (20); Bally, P. R. & Carter 16448 (K) (17); Bally, P. R. O. & R. Melville 15841 (K) (13); Balsinhas, A. 152 (K) (13); Balsinhas, A. & L. Marrime 379 (K) (4); Balslev, H. 447 (MO) (17); Barbey 1051 (US) (13); Barbosa, G. & R. Corrsia 8937 (K) (4); Barter, C. 136 (GH) (16), 158 (GH) (19), 530 (ODU) (17), 1169 (GH) (6f), 1170 (GH) (6a), 1263 (GH) (6e), 1821 (GH) (16); Basera, C. 343 (K) (13); Bashir, M.O. s.n. (K) (11); Batten-Poole, W.H. 226 (K) (23), 371 (K) (11); Battiscombe, E. 495 (K) (16); Batty, M. 131 (K) (27), 870 (K) (27); Baum, H. 490 (K) (10); Baur, R. 34 (K) (10); Bayliss, L. P. 11866 (K) (27); Bayliss, R. D. 3080 (US) (10), 3158 (GH) (13a), 4526 (GH, MO) (10), 5212 (GH) (13a), 7468 (MO) (10), 8112 (MO, US) (6b); Beckett, J. J. 747 (K) (13); Beesley, J. S. S. 252 (K) (10); Belle, E. R. 196 (GH) (17); Ben, V. D. 2441 (K) (6e); Bequaert, C. 35 (GH) (23); Berhaut, R. P. 1630 (K) (6f); Beshir, B. 113 (K) (16), 151 (K) (13), 507 (K) (16), 508 (K) (16), 509 (K) (16), 510 (K) (16), 511 (K) (16); Best, E. B. 52 (K) (11), 74 (K) (17); Bidgood, & J. Lovet 38 (K) (17); Bidgood, et al. 65 (K) (17), 429 (K) (17), 644 (K) (17), 1204 (K) (13); Biegel, H. M. 1912 (K) (4); Bigger, M. 1923 (K) (10); Bingham, M.G. 1178 (MO) (10); Birkett-Smith 49 (K) (26); Bittrimiwx 40 (K) (4); Bjronstad, A. 1294 (K) (4), 1363 (K) (13), 1605 (K) (17); Blackburn, 1863 (GH) (4); Blackmore, et al. 453 (K) (10); Blake, M. 2282 (K) (13); Boaler, S. B. 527 (K)

(17); Bogdan, 3270A (K) (20), 4535 (K) (16), 4600 (K) (11), 4827 (K) (11); Boicher, E. 1841 (GH) (16); Boland, B. G. 743 (K) (16); Boughey, A. S. 14298 (K) (7), 18052 (K) (1), 18067b (K) (1), 180995 (K) (1); Boulos, L. 10250 (K) (28), 10316 (K, MO) (28), 10509 (K) (28); Bouner, E. 1846 (K) (16); Bounougou, E. 126 (K) (24); Boutique, 308 (K) (16); Bousique, R. 168 (K) (22); Bowson, W. J. 452 (K) (16); Box, R. D. 291 (K) (17), 293 (K) (16); Breteler, F. J. 258 (K) (19), 2565 (K) (23), 7110 (K) (7); Britton, L. L. 4778 (BRE) (18); Broadbent, J. A. 158 (K) (13); Brockman, D. 321 (K) (13); Brod 10503 (K) (11); Brodhursk-Hill 176 (K) (17); Brookes, A. J. 4 (K) (23), 19 (K) (16); Brown, A. J. 1644 (K) (13); Brown, E. 21 (K) (17), 238 (K) (17); Brown, F. 91 (K) (13); Brown, L. 1732 (K) (11), 2027 (K) (6a), 2066 (K) (11), 2185 (K) (13); Brown, R. C. 8282 (K) (13); Brown, & Shapiro 4112 (K) (6b); Bruce, E. M. 841 (K) (4); Brummit R. K. 8428 (K) (17), 8661 (K) (4), 18348 (K) (13); Brummit, R. K. & E. A. Banda 8527 (K) (4), 9838 (K) (14); Brummit, R. K. et al. 13988 (K) (6b), 14239 (K) (10), 14911 (K) (4), 16199 (K) (6e); Brunt, M. 404 (K) (17), 1389 (K) (16); Buchanan 1141 (US) (13), 1174 (K) (27); Buhrmann, A. E. 14 (K) (6b); Bullock, A. A. 2212 (K) (6b), 2293 (K) (4), 2391 (K) (4), 3748 (K) (11), 3770 (K) (4), 3826 (K) (13), B14 (K) (17); Bunke, R. 369 (GH) (10); Burbridge, K. 510 (K) (1), 534 (K) (23); Burchell, W. J. 396 (K) (6b), 2482 (K) (10); Burger, W. 914 (K, US) (13), 2846 (K, US) (13), 3204 (K) (13), 3229 (K) (13), 3489 (K) (13); Burke 443/3591 (K) (10); Burnett, G. F. s.n. (K) (11); Burtt, B. D. 1799 (K) (10), 2105 (K) (17), 2423 (K) (16), 2443 (K) (4), 2448 (K) (16), 2732 (K) (6e), 3770 (K) (13), 5095 (K) (13), Bush, R. P. 23 (K) (17).

Carmichael, 390 (K) (10); Carpenter, S. W. 192 (K) (6f); Carter, S. 864 (K) (13), 870 (K) (13); Carter, S. et al. 2251 (K) (13), 2479 (K) (13); Cathorold, 210 (K) (13); Cecil, E. 128 (K) (17), 159 (K) (6b), 247 (K) (27), 259 (K) (10); Chafer, P. A. & A. Nuvunga 6644 (MO) (6b); Champion, A. M. T187 (K) (13); Chancellor, R. J. 5 (K) (17), 56 (K) (16), 72 (K) (11); Chandler, 106 (K) (16); Chapman, J. D. 2935 (K) (11), 3198 (K) (16), 70986 (K) (16); Chapman, J. P. H/579 (K) (13); Chapman, J. W.F. 64 (K) (11); Chapman, & E. G. 7186 (K) (3), 9000 (K) (3); Chase, N.C. 666 (K) (10), 1423 (K) (6b), 6382 (K) (10), 6819 (K) (13), 6853 (K) (13), 6854 (K) (3), 7925 (K) (6b); Cheesman, E. 16 (BM) (10); Chevalier, B. 22.404 (K) (26); Chikawa, I. 676 (K) (13); Choner, H. K. 2005 (K) (4); Cleghorn, W.B. 2357 (K) (3); Codd, L. E. 3729 (K) (13), 9661 (K) (6b); Cole, N. H. 199 (K) (1); Collenette, C. N. 3 (K) (1); Cook, C. D. 358 (K) (5), 386 (K) (11), 445 (K) (6e), 477 (K) (11); Cooper, T. 1349 (K) (10), 2829 (K) (6b); Correia, F. & A. Marques, 4142 (K) (4); Croat, T. B. 53417 (K, MO) (16); Croix, I. F. 2735 (MO) (27); Cruse, A. W. 121 (K) (6b), 372 (K) (6b); Cullett, J. B. 3243 (K) (10); Cunningham, T. F. 18 (K) (10); Curle, N. 98 (K) (16); Curtis, A. G. 707 (GH) (4), 860 (GH) (4), s.n. (GH) (22).

Dacremont 128 (K) (17), 129 (K) (17); Dale, J. R. 252 (K) (13); Dalziel, J. M. 109 (K) (11), 110 (K) (24), 167 (K) (11), 168 (K) (9), 169 (K) (23), 171 (K) (16), 300 (K) (13), 355 (K) (24), 357 (K) (13), 407 (K) (6f), 711 (K) (23), 911 (K) (23), 1912 (BM) (23), s.n. (K) (7); Damwell et al. 252 (K) (13); Daramola, B. O. 62346 (K) (13); Davey, 27126 (K) (16); Davey, J. T. s.n. (K) (13); Davies, R. M. 2890 (K) (13); Davis, P. 53515 (BM) (13), 55245 (BM) (13); Davis, P. & J. Davis 48448 (BM) (13); Davis, R. L. 2 (K, US) (16), 6 (K) (4), 255 (K) (24); Davy, J. B. 932 (K) (6b); Dawe 120 (K) (16), 444 (K) (16); Dawson, W. J. 533 (K) (6e); De Bell, G. S. 878 (K) (4); de Koning, J. 7703 (K) (3); De Lemes, F. & A. Balsinhas 92 (K) (4); de Menezes, A. 377 (K) (10); de Wilde, J. J. 6399 (MO) (6e), 7223 (K) (28); de Wilde, W. J. 4585 (MO) (23); de Wilde, W. J. & B. E. de Wilde-Duyfjes 2347 (K) (22), 2397 (K) (17), 2954 (K) (11), 3471 (K) (13), 9693 (K) (16), 29629 (K) (13); de Winter, B. 3994 (K) (10), 4087 (K) (10); de Witte, G. F. 3332 (K) (17); Decary, R. 9014 (US) (4); Deighton, F. C. 4494 (K) (23), 5146 (K) (1), 6096 (K) (23); Descoings 12466 (P) (8); Dieterlen, M. 527 (K) (6b), 529 (K) (6b); Dinter, K. 5749 (GH) (4); Dowson, W. J. 674 (K) (11); Draper, 25 (K) (11); Drege, J. F. 92713 (MO) (10), 832608 (MO) (6b), 832649 (MO) (6b); Drummond, R. B. 5503 (K) (13); Drummond, R. B. & A. J. Cookson 6185 (K) (6b), 6451 (K) (6b); Drummond, R. B. & J. H. Hemsley 3319 (K) (13), 3721 (K) (13), 3839 (K) (4), 4086 (K) (20), 4247 (K) (20); Duben, W.V. 1845 (S) (4); Dummer, 2629 (US) (22); Durham, F. R. 26/2/24 (K) (17); Dyer, & Merdoom 3421 (K) (11); Dynes, H. 120A (K) (17).

Easterhuper, E. 972 (K) (10); Ecklon, & Zeyher 3.10 (MO) (66), 10.11 (GH, MO, US) (6b); Edwards, L. C. 1923 (K) (16); Eggeling, W. J. 350 (K) (6e), 785 (K) (16), 4033 (K) (6a); Ejiofor, M. C. 30240 (K) (16), 30811 (K) (16); Elhadi, N. & A. Khattab 90/188 (S) (4); Elliott, G. S. 128 (K) (16), 1481 (K) (6a), 4174 (K) (23), 4279 (GH) (6f), 5207 (K, MO) (6f), s.n. (K) (6a); Ellis, P. E. 272 (K) (28); Ern et al. 406 (K) (16), 1477 (K) (16), 1478 (K) (13), 1634 (K) (5); Espirito Santo 2796 (K) (16), 2960 (K) (16), 3199 (K) (21), 3520 (K) (16), 3622 (K, MO) (19), 3636 (K) (23), 3643 (MO) (23), 3668 (K, MO) (19), 3754 (K) (19), 3808 (K, MO) (19), 3824 (K, MO) (23); Esterhinper, E. E. 970 (K) (6b); Evan, I. & J. Erens 1645 (K) (16); Evans-Pritchard, E. E. 27 (K) (16); Eviard, C. 2231 (K) (17); Exell, A. & F. Mendonca 1353 (BM) (2); Exell, A. et al. 214 (BM) (10), 1241 (K) (6b); Eyles F. 985 (K) (13), 1274 (K) (13), 2015 (K) (6b), 8799 (K) (3).

F.A.M. & Hooker, T. 474 (K) (23), 287A (K, MO) (23); F.B. 6431 (K) (4); Faad, G. A. 1881 (K) (16); Faden, A. J. 77/309 (US) (13); Faden, A. J. & R. B. 74/744 (K) (20), 74/1122 (K) (13); Faden, A. J. et al. 74/572 (K) (17); Fames, E. 1905 (K) (11); Fanshawe, D. B. 615 (K) (6b), 2083 (K) (13), 4689 (K) (23), 4920 (K) (6b), 5439 (K) (13), 5543 (K) (13), 5819 (K) (6b), 10003 (K) (11); Farmer, L. 131 (K) (13); Farris, J. 1736 (K) (17); Fauckner, W. 3721 (K) (11), 4877 (K) (13); Fauckon, D. 844 (K) (4); Fauckor, H. 3605 (K) (4); Fauckorn 2494 (K) (4), Faulkner, H. G. 90 (K) (4), K118 (K) (27), A376 (K) (2), 661 (K) (27); Fay, J. M. 1896B (K) (22), 2787 (K) (17), 2794 (K) (5), 2867 (K) (17), 2877 (K) (17), 3354 (K) (13), 4083 (K) (23), 5066 (K) (17), 5067 (K) (17), 5068 (K) (17), 5069 (K) (17), 5479 (MO) (6e), 6021 (K, MO) (19), 7057 (MO) (6e); Fay, J. M. & J. Doka 5070 (K) (16); Fitzgerald, D. V. 3235 (K) (17); Forbes, H. W. 1/1929 (K) (4); Fotius 2177 (K) (24), 2219 (K) (6d); Friis et al. 918 (K) (25); Frith, A. C. 147 (K) (5); Froment, D. 588 (K) (16); Fyffe, R. 32 (K) (16), 164 (K) (16).

Gaepin 1709 (US) (11); Gairdner, A. E. 194 (K) (13), 412 (K) (17); Galpin, E. E. 9768 (K) (6b), 13131 (K) (6b); Gande, P. 39/74 (K) (11); Gardner, H. M. 3517 (K) (13); Gaughan, J. H. 1614 (K) (11); Gbesofssiere 3724 (GH, K) (16); Gbile et al. 63394 (K) (13), 64088 (K) (16), 72899 (K) (16); Gbile & B. O. Daramola 245 (K) (23); Geerling, C. & J. Bokdam 934 (K) (16), 2029 (MO) (6f); Geilinger 58 (K) (27), 499 (K) (27), 585 (K) (4), 2420 (K) (17), 4788 (K) (17) ; Germain, R. 4269 (K) (17), 5717 (K) (13), 6666 (K) (11), 6747 (K) (11); Germishuizen, G. 1018 (K) (6b), 3534 (K) (18); Gerrad, W. T. 424 (K) (11); Gerstner, J. 7151 (K) (4); Gilbert, J. B. 4253 (K) (13), 4347 (K) (13), 4722 (K) (13), 4723 (K) (13), 4918 (K) (13), 4938 (K)

(13), 13104 (K) (13), 17889 (K) (3); Gilbert, J. B. & F. Kariuki 18863 (K) (6e); Gilbert, J. B. & S. P. Kibuwa 19932 (K) (11); Gilbert, M. G. 1663 (K) (17), 2412 (K) (13), 4071 (K) (13), 4072 (K) (28), 4840 (K) (17), 6215 (K) (22), 6268 (K) (13); Gilbert, M. G. & A. Getachew 2958 (K) (13); Gilbert, M. G. & S. B. Gilbert 1078 (K) (13), 1250 (K) (6e), 1408 (K) (28), 1438 (K) (13); Gilbert, M. G. & T. G. Jefford 4594 (K) (20); Gilbert, M. G. & M. Thulin 415 (MO) (6e), 708 (K) (17); Gilbert, M. G. et al. 24 (K) (13), 304 (K) (13), 320 (K) (17), 345 (K) (13), 417 (K) (13), 524 (K) (13), 525 (K) (13); Gilbert, S. B. 1122 (K) (20); Gilger, W. 710 (K) (11); Gille 39 (K) (17); Gillespie, J. B. 186 (K) (4), 246 (K) (4); Gillett, J. B. 16369 (K) (16), 17889 (MO) (27); Gillett, J. B. & R. Faden 18152 (K) (13); Gillett, J. B. et al. 5045 (K) (17), 25031 (K) (13), 25136 (K) (13); Gilliland, H.B. k1398 (BM, K) (10); Gkvile, J. W. 41 (K) (4); Glanville, R. R. 241 (K) (6f); Gledhill, D. 285 (K) (6f), 328 (K) (23), 942 (K) (23); Glover, P. E. & H. B. Gilliand 1231 (BM, K) (20); Glover, P. E. & Samuel 2722 (K) (17); Glumptre, E. D. 25 (K) (23); Gooddny, H.V. 101 (K) (13); Goodies, R. 69 (K) (13); Gossweller, J. 199 (K) (16), 329 (K) (13), 558 (BM) (23), 647 (K) (13), 1466 (K) (4), 2234 (K) (6b), 2916 (K) (17), 3504 (K) (2), 3540 (K) (13), 4545 (K) (23), 14219 (K) (1); Goyder, D. J. & A. J. Paton 3515 (K) (11); Goyder, D. J. & A. R. Smith 3216 (K) (13); Goyder et al. 3631 (K) (17); Graham, M. A. 2405 (K) (6e); Graham, M. D. 3031 (K) (13); Graham, R. M. 272 (K) (4), 497 (K) (13), 1577 (K) (27), 1675 (K) (13), 1701 (K) (22), 2154 (K) (22); Grandraux, L. A. & F. Delemos 7913 (K) (11); Grant, A. L. 3141 (MO) (6b), 3505 (MO) (10), 3570 (MO) (6b), 2212387 (MO) (10); Greenstock, W. 1613834 (MO) (10); Greenway, P. J. 1204 (K) (4), 2274 (K) (17), 4358 (K) (13), 4462 (K) (6e), 5097 (K) (4), 5330 (K) (27), 5371 (K) (17), 7392 (K, US) (16), 7473 (K) (6e), 7840 (K, S) (10), 10519 (K) (4), s.n. (K) (13); Greenway, P. J. & J. M. Brenan 8095 (K) (6b), 8213 (K) (6b); Greenway, P. J. & Kanuri 11245 (K) (13), 11870 (K) (13), 14220 (K) (4); Greenway, P. J. & R. M. Polhill 11305 (K) (4), 11736 (K) (4); Greenway, P. J. & S. P. Rawlins 9463 (K) (11); Grosvenor, R. K. 144 (K) (13); Greenway, P. J. et al. 13160 (K) (13), 14082 (K) (13); Grosvenor, R. & J. Renz 1038 (K) (3); Guerra, J. A. 3812 (K) (11); Guile, D. P. 1662 (MO) (23).

Haarer, Q. E. 177 (K) (13), 338 (K) (17), 517 (K) (6e), 596 (K) (17), 637 (K) (10), 1767 (K) (17), 2307 (K) (11), 5127 (K) (13), 5128 (K) (13), s.n. (K) (17); Hagerup, O. 478 (K) (16); Hakki, et al. 289 (K) (6e), 309 (K) (6e), 1504 (K) (24), Hale, M. 105 (K) (20); Hall, J. B. 90 (K) (11), 131 (K) (6f), 260 (K) (6f), 400 (K) (16), 452 (K) (5), 558 (K) (11), 1392 (K) (6e), 1998 (K) (26), 2028 (K) (24), 2058 (K) (13), 3079 (K) (5), s.n. (K) (7); Hall, J. B. & B. O. Daramola 67404 (MO) (6e); Hall, J. B. & Swaine GC46220 (US) (24); Halle, N. & Villiers 4914 (P) (15); Hambler, D. J. 768 (K) (23); Hansen, O. J. 854 (K) (20), 3016 (K, MO) (6b), 3375 (K) (13); Hardy, D. S. 961 (K) (13); Hardy, D. S. & P. R. O. Bally 10826 (K) (13); Harison, 2 (K) (11); Harlan, H. V. 1029870 (US) (16); Harold, J. M. 903 (K) (10); Harrer, A. E. 2227 (K) (6a); Harris, B. J. 1774 (K) (13), 2523 (K) (11); Harris, B. J. & A. McCusker BJH2568 (MO) (27); Harris, E. 4 (K) (7), 5 (K) (16), 6 (K) (23), 7 (K) (7), 8 (K) (23), 9 (K) (16), 10 (K) (19), 11 (K) (6f); Harris, L. D. 9 (K) (20); Harris, T. M. 195 (K) (7), 786 (S) (27); Hayes, T. R. 539 (K) (6f); Haygarth, W. 92714 (MO) (10); Hazel, C. 289 (K) (16); Hazel, G. 442 (K) (11); Hazel, O. 521 (K) (17); Hedbert, O. 7022 (UPS) (4); Hemming, C. F. & R. M. Watson 30216 (K) (13); Henriques, C. 853 (K) (17); Hepper, F. N. 1162 (K) (16), 1218 (K) (16), 1278 (K) (16), 1392 (K) (23), 1397 (K) (19), 1847 (K) (16), 2745 (K) (17), 3721 (K) (16), 3759 (K) (16), 3915 (K) (23), 7333 (K) (3), 7368 (K) (3), 7373 (K) (4), 7377 (K) (13); Hepper, F. N. & D. V. Field 5218 (K) (4); Hepper, F. N. & A. Jaeger 6618 (K, MO) (10); Hepper, F. N. & J. K. Morton 2314 (K) (6e), A3039 (K) (6f), A3050 (K) (6e), A3110 (K) (6f), A3160 (K) (16); Hettewy, A. W. 52 (K) (13), 179 (K) (13), 186 (K) (13); Hildebrandt, J. M. 1907 (UPS) (27); Hill, A. W. 51 (K) (6f); Hill, B. 755 (K) (6a); Hilliard, O. M. & B. L. Burtt 7861 (MO) (10), 10012 (MO) (6b), 12139 (K) (10); Hindort 818 (K) (16); Holub, E. 1834 (K) (10), 1883 (K) (11), 1993 (K) (13); Hooper, S. S. & C. C. Townsend 503 (K) (6b); Hopkins, J. C. 9712 (K) (11), 14555 (K) (13); Hornby, R. M. 2898 (K) (11); Hossain, & Agyakwah 37826 (K) (5), 37829 (K, S) (5), 37831 (K) (13), 37954 (K) (16), 37855 (K) (16), 37957 (K, US) (16), s.n. (K) (5); Howaiduud 1599 (K) (22); Hudson, D. 64 (K) (17), 296 (K) (17), 2566 (K) (17); Hughes, N. K. s.n. (ODU) (10); Huntley, B. J. 192 (MO) (6b); 2033 (MO) (10); Hutchinson, J. 2381 (K) (6b), 2586 (K) (6b), 2854 (K) (10); Hutchinson, J. & J. B. Gilbert 3537 (K) (3); Hynes, A. 478 (K) (17), 576 (K) (16).

Innes, R. R. 373 (K) (27), 909 (K) (6f), 30116 (K) (16), 30748 (K) (16); Irvine, R. 146 (K) (6e), 466 (K) (6e), 1426 (K) (6e), 1620 (K) (6e), 4534 (K) (16), 4572 (K) (13), 4574 (K) (13), 4581 (K) (16), 4667 (K) (5), 4668 (K) (7), 4669 (K) (7), 4732 (K) (7), 4914 (K) (5), 4915 (K) (6e), s.n. (K) (6e); Ithwa, R. 1370 (US) (16); Iverson, et al. 87612 (K) (13).

Jackson, G. 649 (K) (6b), 1656 (K) (4), 2282 (K) (3), U103 (K) (17); Jackson, J. A. D. 119 (K) (16), 254 (K) (11); Jackson, J. K. 2722 (K) (13), 3196 (K) (17); Jackson, W. B. 415 (K) (17); Jacques-Felix, 1241 (P) (21); Jaeger, H. D. 1225 (K) (11); Jaeger, P. 753/51 (K) (6c), 790 (K) (23), 2541 (K) (6c); Jahandiez, E. 149 (BM) (13), 202 (BM) (13); James, E. 1905 (K) (11), 1905 (K) (16), 1905 (K) (17); Jansen, P. C. 8040 (K) (13); Jarrett, T. 103 (K) (6e); Jasen, P. C. 8158 (K) (4); Jeagu, A. J. 84 (K) (4); Jeffery, G. M. K242 (K) (4); Jefford, et al. 53 (K) (13); Jex-Blake, M. 6880 (K) (20); Joanna, B. 8928 (K) (20), 8947 (K) (13); Jocobsz, M. L. 218 (K) (10), 2011 (K) (6b); Johannes, 26–1–22 (US) (10); Johnson, W. P. 32 (K) (13), 101 (K) (4); Johnston, A. H. 83 (K) (11); Johnstone, M. 1551 (K) (17); Jones, A. 3660 (K) (16), 7164 (BM) (23); Jones, K. W. 41 (K) (4), 501 (K) (16), 502 (K) (16), 503 (K) (16), 504 (K) (16), 505 (K) (16), 506 (K) (16), 512 (K) (16), 513 (K) (16), 514 (K) (16), 515 (K) (16), 516 (K) (16), 517 (K) (16), 518 (K) (16), 519 (K) (16), 521 (K) (16), 522 (K) (16), 523 (K) (16), 524 (K) (16), 525 (K) (16), 526 (K) (16), 527 (K) (16), 528 (K) (16), 529 (K) (16), 530 (K) (16), 531 (K) (16), 532 (K) (16), 533 (K) (16), 534 (K) (16), 535 (K) (16); Jones, V. 3 (K) (11); Jordan, H. D. 152 (K) (23), 190 (K) (6f); Junod, H. A. 276 (BRE) (18).

Kaessner, T. 474 (K) (20), 645 (BM, K) (20), 1474 (BM) (20); Kassner Exped., 190 (K) (17), 2397 (K) (6b), 2786 (K) (16), 3148 (K) (16); Keay, R. W. J. 21674 (K) (16), 25446 (K) (13), 25833 (K) (6f), 25896 (K) (6e), 25977 (K) (24), s.n. (K) (6f); Kelly, R. D. 80 (K) (10); Kemp, S. 701 (MO, US) (6b); Kennedy, J. D. 2894 (K) (16); Kenyon, E. 1901 (K) (13); Kerfoot, O. 1313 (K) (13), 3815 (K) (13); Keritochonorag, H. 2166 (K) (16); Kers, L. E. 1399 (S) (24); Kersting, 665 (K) (7); Kesollesen, 2817 (K) (27); Kew, J. 19911 (MO) (27); Kilian, N. 1833 (K) (13); Killick, D. J. 3857 (K) (6b); Killick, H. J. 234 (K) (11); Killick & Leisner 3415 (K) (11); Kimani, Z. J. 186 (K) (13); King, A. E. 260 (K) (11); King, E. L. 175 (K) (23),

175B (K) (23); Kirk, J. 15/1/63 (K) (11), 2179 (K) (17), 9/68 (K) (4), 1 (K) (13); Knepper, D. K. 8/22/89 (ODU) (13h), 8/24/89 (ODU) (13h), 8/25/89 (ODU) (17), 8/26/89 (ODU) (13d), 8/26/89 (ODU) (16), 8/26/89 (ODU) (5), 8/25/89 (ODU) (5), 9/20/89 (ODU) (16), 9/22/89 (ODU) (16), 9/23/89 (ODU) (16), 9/23/89 (ODU) (5), 9/24/89 (ODU) (16), 9/26/89 (ODU) (5), 9/19/89 (ODU) (13g), 9/21/89 (ODU) (5), 9/23/89 (ODU) (5); Konging, J. de & D. Zunguze 8038 (K) (6b); Korans, J. 1508 (K) (10), 1596 (K) (13), 3060 (K) (6b), 3299 (K) (6b), 3364 (K) (13); Krauss, J. 213 (MO) (6b); Koritschoner, H. 2059 (K) (11); Kuchar, P. 15312 (K) (13); Kuntze, O. 7/iv94 (US) (4), 94 (K) (13), 1913 (K) (4).

La Croiz, I. F. 2735 (MO) (3); Laferrere, M. 112 (K) (26); Lamb, P. H. 4 (K) (16); Lambrecht, D. 479 (K) (11); Lambrecht, F. L. 31 (K) (13), 264 (K) (6b), 440 (K) (6b); Lamprey, H. F. 515 (K) (6e); Lanbrester, C. H. 18 (K) (6a); Langdale-Brown, I. 2392 (K) (16), 2421 (K) (17); Last, J. T. 1887 (K, US) (4), 1908 (K) (17); Latilo, M. G. 18214 (K) (16); Latilo, M. G. & B. O. Daramola 28792 (K) (16); Lavranos, J. J. 4265 (MO) (10), 7394 (K) (20); Lavranos, J. J. & Cater 23192 (K) (13); Lavranos, J. et al. 24938 (K) (13); Lawlor, D. W. & B. J. Hall 28 (K) (11), 34 (K) (24), 73 (K) (24), 461 (K) (24), 468 (K) (13), 508 (K) (7), 46508 (K) (5); Lawrence, E. 15 (K) (4), 28 (K) (4), 163 (K) (4); Lea, A. LR20 (K) (11); Lea, J. D. 10 (K) (27), 17 (K) (16), 58 (K, ODU) (24), 180 (K) (24); Leach, L. C. 9189 (K) (27), 11232 (K, MO) (27); Leach, L. C. & R. F. Smith 11011 (K) (27); Lean, O. B. 37 (K) (11); Lebrun, J. 1759 (GH) (16), 3374 (GH, K) (16), 3535 (MO) (13), 3557 (GH) (11), 9631 (K) (16); Lecard, F. s.n. (K) (16); Ledermann, C. 4529 (BM) (6d); Lee, J. l6 (K) (11); Leeuwenberg, A. J. 4357 (K, MO) (6f), 10189 (K) (16); Leighton, J. M. 2948 (MO) (6b); Leistner, O. A. 1817 (K) (13); Lelehase, N. 3673 (MO) (10), 3764 (MO) (10); Lely, H.V. 146 (K) (6e), 255 (K) (11), 489 (K) (13), 535 (K) (13), 590 (K) (16), 680 (K) (19), P83 (K, MO) (6f), P369 (K) (11), P567 (K) (7), P785 (K, MO) (23); Lester, J. B. 34 (K) (23); Lester, R. N. & M. Morrison, 2337 (K) (6a); Letouzey, R. 624 (K) (6f), 6299 (K) (11), 6406 (K) (16), 7209 (K) (16); Levy, B. 1177 (K) (13); Lewalle, H. J. 335 (K) (11), 1474 (K) (13), 2402 (K) (17), 10634 (BM) (13); Liben, L. 976 (K) (13), 2038 (K) (11), 2401 (K) (4), 3705 (K) (11); Liebenberg, L. C. C. 1029 (K) (16), 1532 (K) (11), 4942 (K) (13), 6904 (K) (6b), 6986 (K) (10), 8581 (MO) (10), 8748 (MO) (10), A516 (K) (13); Lind, E. 340 (K) (6a), 387 (K) (16), 2027 (K) (6a); Linder, D. H. 1927 (GH) (22), 1966 (K) (13); Linoblom, K. G. 1911 (S) (10); Lisowski 80551 (BR) (6c), 80579 (BR) (6c); Lloyd, H. 80 (K) (10); Lobin, W. 6687 (K) (13); Lock, J. M. 81/77 (K) (13), 81/132 (K) (5), 81/247 (K) (17), 81/248 (K) (24), 81/2658 (K) (13), 82/31 (K) (11), 82/44 (K) (13); Longnot & Verdcourt 3200 (K) (6e); Looe, P. P1048 (K) (17); Lord, J. K. 8169 (K) (13); Lovett, J. 1612 (K) (13), 1637 (K) (4); Lovett, J. & C. Congdon 1404 (K) (13), 1931 (K) (13); Lowe, J. 1379 (K) (16), 1484 (K) (23), 1671 (K) (16), 2760 (K) (16), 3378 (K) (6f), 4463 (K) (23), 4828 (K) (16), A12 (K) (13); Lugard, E. J. 127 (K) (13), 164 (K) (13); Luke, W. R. 2459 (K) (11), 3054 (K) (13), 3390 (K) (13), 3489 (K) (20); Lye, K. A. 4747 (K) (11), 5379 (K) (13), 9078 (K) (13), s.n. (K) (17); Lynes, H. 18 (K) (10), 53 (BM) (13), 577 (BM) (11), 578 (BM) (24).

Mabberley & McCall 86 (K) (17); Macaulary 867 (K) (6b), 927 (K) (6b); MacCintosh, D. G. 103 (K) (16); MacDonald, W. 21 (K) (13); MacOwan, L. 426 (GH) (10); Magaji, S.O. M6660 (K) (16); Magogo, F. C. 373 (K) (27); Magogo, F. C. & R. Estes, 1152 (K) (17), 1152 (K) (22), 1153 (K) (17), 1153 (K) (22); Magogo, F. C. & P. Glover 1050 (K) (27), 1089 (K) (4); Magogo, F. C. & R. R. Innes 849 (K) (27); Maitland, J. 103 (K) (11), 1926 (K) (16), 1946 (K) (16), s.n. (K) (11), s.n. (K) (16); Mansfield, R. A. 9/4/78 (ODU) (22), 9/4/78 (ODU) (7); Marmo, V. 36 (K) (23); Masierson, G. 217 (K) (3); Massey, R. E. 47 (K) (16); Mathew, B. 6126 (K) (6e); Mauteiro 6193 (K) (13); Mavi, S. 626 (K) (6b); McCintosh, D. G. H. 3k (K) (17), 57k (K) (11), 109k (K) (11); McClean, A. 595 (K) (6b); McClean, B. 209 (K) (6b); McClounie, T. 60 (K) (4), 178 (K) (3); McDonald, J. 910 (K) (16); McGillett 1007 (K) (13); Mearns, E. A. 2885 (GH) (11), 3005 (GH) (16); Meel, L. V. 1432 (K) (17); Meikle, R. D. 746 (K) (16), 768 (K) (23), 1011 (K) (6a); Melville, F. A. & T. Hooker 287a (K, MO) (23); Mesfin et al. 4058 (K) (6e); Methew, L. 162 (K) (6b), 257 (K) (27); Meurillon, A. 1363 (K) (5), CNAD577 (K) (23); Meurillon, A. et al. 1976 (K) (16); Meyer, F. G. 7594 (K, US) (16), s.n. (K) (16); Meyers, J. G. 7329 (GH, K) (5), 7867 (GH) (19); Mgaza, C. D. 434 (K) (17); Michael 9531 (K) (6d), 9706 (K) (6d); Michel, B. 13 (K) (11); Michel, G. 5367 (K) (4); Migeod, F. W. 13 (BM) (27), 35 (BM) (27), 50 (BM) (27), 730 (BM) (27); Mildraed, 1–232 (K) (17), 9501 (K) (17); Miller, H. 3/1896 (K) (23); Miller, O. B. 1590 (MO) (6b), 1939 (K) (13), 2630 (K) (6b), 2671 (K) (6b), 4827 (K) (6b), 5737 (K) (6b), 7260 (K) (6b); Miller et al. 615 (BM) (13); Milne-Redhead, E. 3577 (K) (6b), 3756 (K) (17), 4018 (K) (10), 4155 (BM, K) (11), 4161 (K) (2), 5043 (K) (6f); Milne-Redhead, E. & B. Mhroro 9693 (K) (4); Milne-Redhead, E. & P. Taylor 882 (K) (17), 8421 (K) (27), 9524 (K) (13), 10018 (K) (13), 10024 (K) (3), 10577 (K) (3); Mitchell, B. L. 24/78 (K) (11); Mitchison, N. 1975 (K) (13), 1975 (K) (4); Mitte 314 (US) (16); Mogg, A. O. 5935 (MO) (6b), 30317 (K) (17); Moggridge, F. Y. 548 (K) (4), 1868 (US) (16); Mohamed, K. I. 10/25/88 (ODU) (13b), 10/26/88 (ODU) (16), 10/26/88 (ODU) (13e), 10/27/88 (ODU) (16); Moiser, B. 63 (K) (13), 9 (K) (16); Moll, E. J. 4723 (K) (6b); Moomaw, J. C. 953 (K) (27); Mooney, H. F. 8427 (K) (17); Moriarty, A. 441 (K) (17), 469 (K) (27); Morton, J. K. 94 (K) (23), 1104 (K) (23), 1677 (K) (13), 2527 (K) (19), 6123 (K) (6e), 6214 (K) (16), 6224 (K) (16), 6292 (K) (19), 7171 (K) (6e), 8794 (K) (6e), 8795 (K) (6f), 9370 (K) (23), A663 (K) (6f), A1394 (K) (16), A2045 (K) (6e), A3016 (K) (6f), A3295 (K) (7), A3488 (K) (6e), A3926 (K) (6e), A4241 (K) (26), GC8601 (K) (26), s.n. (K) (5), s.n. (K) (23); Morton, J. K. & J. M. Dalziel 145 (K) (6e); Morton, J. K. & D. Gledhill 1093 (K) (1), 3035 (K) (19), 18771 (K) (1); Mouculie, A. 574 (K) (13); Mouteiro 6143 (K) (23); Mugwedi, E. 1371 (K) (4); Muller, P. J. 2205 (K) (6b); Murray, A. L. 426 (K) (10); Musselman, L. J. 124 (K) (11), 198 (K) (24), 6118 (ODU) (7), 6122 (ODU) (5), 6123 (ODU) (5), 6124 (ODU) (5), 6131 (ODU) (7), 6139 (K, ODU) (16), 6140 (ODU) (16), 6143 (ODU) (16), 6144 (K, ODU) (16), 6145 (K, ODU) (16), 6152 (ODU) (16), 6154 (K) (16), 6155 (K) (16), 6162 (K, ODU) (16), 6174 (ODU) (13), 6180 (ODU) (13a), 6212 (K) (16), 6231 (K) (24), 6256 (K) (24), 6260 (K) (17), 6269 (ODU) (13d), 6279 (K, ODU) (16), 7003 (ODU) (4), 7011 (ODU) (6b), 7015 (ODU) (4), 7022 (ODU) (4), 7030 (ODU) (10), 7032 (ODU) (6b), 7038 (ODU) (10), 7039 (ODU) (10), 7040 (ODU) (6b), 7060 (K, ODU) (5), 7061 (ODU) (24), 7064 (ODU) (13c), 7085 (K, ODU) (5), 7086 (ODU) (11), 4/10/86 (ODU) (11), 4/9/86 (ODU) (11), 8/23/86 (ODU) (17), 8/23/86 (ODU) (13c), 8/23/86 (ODU) (24), 8/23/86 (K) (24), 10/13/88 (ODU) (5), 10/13/88 (ODU) (11), 10/16/88 (ODU) (19), s.n. (K) (24), s.n. (K) (17); Musselman, L. J. & R. A. Mansfield 5522 (ODU) (23), 5524 (ODU) (5), 5527 (ODU) (16), 5533 (ODU) (19),

5535 (ODU) (16); Musselman, L. J. & K. I. Mohamed 9/30/88 (ODU) (6e), 9/30/88 (ODU) (7), 10/1/88 (ODU) (5), 10/1/88 (ODU) (7), 10/1/88 (ODU) (13h), 10/1/88 (ODU) (16), 10/1/88 (ODU) (17), 10/2/88 (ODU) (5), 10/2/88 (ODU) (7), 10/2/88 (ODU) (17), 10/2/88 (ODU) (22), 10/4/88 (ODU) (5), 10/4/88 (ODU) (7), 10/4/88 (ODU) (17), 10/4/88 (ODU) (22), 10/4/88 (ODU) (24), 10/5/88 (ODU) (5), 10/5/88 (ODU) (22), 10/6/88 (ODU) (5), 10/6/88 (ODU) (7), 10/6/88 (ODU) (13c), 10/8/88 (ODU) (5), 10/8/88 (ODU) (17); Musselman, L. J. & J. H. Visser 4/2/86 (ODU) (13); Musselman, L. J. et al. 7 (K) (19), 064 (ODU) (13c), 7063 (ODU) (24), 7066 (ODU) (13c), 4/9/86 (ODU) (11), 4/17/86 (ODU) (4), 9/4/86 (ODU) (4), 10/13/88 (ODU) (17), 10/13/88 (ODU) (5), 10/16/88 (K, ODU) (6d), 10/16/88 (ODU) (23), 10/16/88 (ODU) (13g), s.n. (K) (19), s.n. (K) (23); Mutch, M. I. 27952 (K) (16); Mutimushi, J. M. 537 (K) (10), 932 (K) (6b); Mwasumbi, L. B. 10487 (K) (11); Myers, J. G. 6656 (K) (17), 6997 (K) (11), 7000 (K) (17), 7329 (K) (5), 7336 (K) (17); Myres, R. F. 294 (K) (17), 303 (K) (6b).

N.D.H 7361 (K) (13); Nafuer, E. R. 5343 (K) (16); Napier, E. R. 51 (K) (4), 957 (K) (20); Napper, D. & R. Faden 1839 (K) (20); Nation, O. 29 (K) (10), 143 (K) (13), 313 (K) (6b); Ncube, M. 75 (K) (11); Nelson, W. 394 (K) (10); Nesbould, J. G. 1958 (K) (4); Newberreu, R. J. 6 (K) (23); Newbould, J. G. B. 1958 (K) (13); Ngoni, J. F. 160 (K) (13), 295 (K) (13), 1249 (K) (6b); Niamir, M. 17 (K) (13); Nicholas, A. & V. D. Berg 1736 (K) (6b); Nicholas, A. & D. Neave 2109 (K) (6b); Nichols, E. H. 11 (K) (23); Niger 495 (UPS) (16); Nilson, J. 332 (K) (6e); Noak, S. L. 311 (K) (10); Noble, H. L. 30 (K) (16), 31 (K) (16); Norlindh, T. & H. Weimarck 4878 (K) (3), 4956 (K) (17), 44347 (K) (6b); Norman, E. M. 294 (K) (16); Norrgrann, G. 459 (K) (4); Nuvunga, A. 471 (K) (3).

Okafor, J. C. 46885 (K) (23); Olmuntus, B. 2507 (K) (6e); Olorunfemi, J. 55046 (K) (16); Olufsen, O. 479 (K) (13), 705 (K) (23), 1927 (K) (23); Onochie, C. F. A. 18697 (K) (6e); Onsow, M. P. 709 (K) (6f); Onwudinjo, D. C. 24005 (K) (11); Ortendahl, V. 487 (UPS) (13a); Ossent, J. 478 (K) (13).

Padva, J. H. 326 (K) (17); Paget-Wilkes, C. F. 700 (MO) (27); Panayotis, L. J. 98 (K) (17); Parker, C. 985 (GH) (23), 1066 (ODU) (11), 1316 (ODU) (13f), 1976 (K, ODU) (5), 1977 (ODU) (5), 1978 (K) (5), 1979 (K) (16), 1994 (ODU) (16), 2023 (K) (13), 2031 (K, ODU) (13d), 2031 (K) (13), 2044 (K) (13), 2046 (K) (5), 2210 (ODU) (11), 2264 (ODU) (13h), 2265 (ODU) (13f), 2306 (ODU) (16), 2312 (ODU) (13d), 2314 (ODU) (5), 2316 (ODU) (16), 2317 (ODU) (16), 2318 (ODU) (5), 2321 (ODU) (16), 2332 (ODU) (16), 2443 (ODU) (4), 2682 (K) (7), 2984 (ODU) (20), 3215 (ODU) (5), 4001 (ODU) (22), 4034 (K, ODU) (20), 4041 (ODU) (17), 4065 (K) (5), 4074 (K) (17), 4075 (ODU) (16), (K) (16), E22 (K) (16), GM/522/H (K) (20); Parkinson, V. 1985 (ODU) (24), 9/18/85 (ODU) (22), 9/17/86 (ODU) (7), A1 (K) (24), A3 (K) (16), A4 (K) (16); Parsons, D. A. L79 (K) (16); Pasch 7846 (K) (16), 7916 (K) (16); Patel, I. H. & W. Nachamba 1775 (K) (11); Paterson, A. 59 (K) (11); Paulo, S. 105 (K) (27); Pawek, J. 1649 (K) (17), 3349 (K) (4), 4112 (K) (27), 5135 (K) (13), 5266 (K) (13), 7799 (K) (6e), 8301 (K) (17), 9499 (K) (17), 9575 (K) (17), 9601 (K) (13), 10942 (K) (17), a12015 (MO) (27), b12015 (K) (3), 12317 (K, MO) (6e), 12566 (K) (4), 13856 (K) (17), 13913 (K) (11), 13954 (K, MO) (11), 14332A (K) (14), 14332B (K) (11); Pearson, H. H. 2038 (K) (13), 2421 (K) (13), 8122 (K) (13); Perceval, A. B. 1901 (MO) (10); Pereira, J. A. 2328 (K) (19), 2368 (K) (19), 2984 (K) (21), 2984 (K) (6a), 3580 (K) (16); Peters, 8 (K) (13); Peterson, C. 823 (K) (17), 833 (K) (10); Petherick 1862 (K) (17), 1863 (K) (16), Petterson, B. 16819 (K) (16); Pfund, J. 86 (K) (24), 679 (K) (16); Philcox, D. & R. B. Drummond 070 (K) (13), 9050 (K) (17), 9054 (K) (3), 9094 (K) (13); Philcox, D. & M. J. Leppard 8574 (K) (13), 8575 (K) (13), 8677 (K) (24), 8718 (K) (13), 8870 (K) (17); Philcox, D. & T. Muller 9080 (K) (6b), 9083 (K) (13); Philcox, D. et al. 8563 (K) (17), 8610 (K) (17), 8748 (K) (13), 8796 (K) (11), 8799 (K) (13), 8933 (K) (10), 9017 (K) (6b), 9019 (K) (10), 9093 (K) (3), 9127 (K) (3), 9873 (K) (4), 9908 (K) (17), 10170 (K) (6b), 10190 (K) (17), 10210 (K) (14); Phillips, E. 661 (GH, MO) (6e), 832 (K, MO) (6e), 1215A (MO) (6e); Phillipson, P. B. 138 (MO) (6b); Phipps, J. B. 342 (K) (6b), 2594 (K) (4); Phiri, R. 79 (K) (4); Pichi-Sermolli, R. 1295 (K) (16), 1296 (K) (13); Pielou, D. P. 130 (K) (13), s.n. (K) (17); Plant, H. 706 (K) (6a); Plowes, D. C. 32013 (K) (13); Pobeguin, M. 364 (K) (6f), 1020 (K) (5); Polhill, C. 496 (K) (17); Polhill, R. & S. Paulo 579 (K) (13), 950 (K) (13); Poulo, S. 105 (K, MO) (27); Powell, J. H. 876 (K) (4); Powys, 197 (K) (10); Prince, S. D. 116 (K) (4), 200 (K) (24), 215 (K) (11), 401 (K) (24); Probegum, M. 364 (K) (6e); Prosser, D. U. 1643 (K) (6b), 1784 (BRE, K) (18); Puff, C. & E. Kelbessa 921220 (K) (20); Puff, C. et al. 8209 (K) (20), 820922 (K) (16); Purseglove, J. W. 487 (K) (16), 560 (K) (16), 1609 (K) (6e), 2053 (K) (11), 2711 (K) (6a), P2424 (K) (17); Pusch, 2689 (K) (4).

Quarre, 1451 (K) (11).

Radjil 594 (GH) (16); Ragslane 1251 (US) (6e); Ramaiah, K.V. 2973 (K) (24); Ranger, G. A. 304 (K) (10); Rax, R. D. 292 (K) (24); Raymond, R. J. 319 (K) (17); Raynal, A. R. 2282 (K) (7), 22057 (K) (7), 22063 (K) (13), 22127 (K) (5), 22139 (K) (7), 22149 (K) (5), 22181 (K) (7), 22194 (K) (16), 22196 (K) (7), 22200 (K) (16), 22210 (K) (16), 22211 (K) (13), 22220 (K) (16), 22221 (K) (16), 22244 (K) (13), 22263 (K) (16), 22277 (K) (24), 22290 (K) (5), 22309 (K) (5), 22394 (K) (5), 22395 (K) (5), 22435 (K) (7), 22526 (K) (16), 22565 (K) (13), 22573 (K) (16), 22581 (K) (5), 22602 (K) (16), 22603 (K) (13), 22613 (K) (13), 22636 (K) (13), 22640 (K) (7), 22641 (K) (24); Read, J. G. 10 (BM, K) (10), 27 (BM, K) (10); Reading University/BM 292 (BM) (13); Red, E. 1424 (K) (17); Reed, S. W. 16290 (K) (11); Reed 14 (K) (16), 3/1888 (K) (4); Reekmans, M. 4257 (K) (17), 6786 (K) (6e), 6926 (K) (13), 7125 (K) (6a), 7166 (K) (6a), 9834 (K) (13), 10314 (K) (17), 23/2/75 (K) (11); Rehmann, A. 4671 (K) (13); Rensburg, H. J. 1516 (K) (13), 2772 (K) (11), 23107 (K) (13); Revell, R. J. 321 (K) (17); Richards, H. M. 657 (K) (13), 668 (K) (11), 676 (K) (6b), 1169 (K) (6b), 1771 (K) (27), 4205 (K) (6b), 4512 (K) (13), 4699 (K) (6b), 4780 (K) (11), 4813 (K) (13), 5230 (K) (4), 5423 (K) (13), 7425 (K) (6b), 9026 (K) (13), 9103 (K) (11), 10542 (K) (6e), 10763 (K) (6b), 10921 (K) (13), 10953 (K) (6b), 12222 (K) (11), 12486 (K) (13), 12570 (K) (6b), 12813 (K) (13), 14824 (K) (11), 14858 (K) (13), 15025 (K) (6b), 16275 (K) (6b), 17147 (K) (6b), 17965 (K) (13), 17980 (K) (4), 19831 (K) (13), 24360 (K) (4), 24897 (K) (20), 25019 (K) (10), 25389 (K) (6e), 25465 (K) (13), 26863 (K) (20), 28461 (K) (13); Richie, A. H. 1924 (K) (4); Riches, C. R. 226 (K) (16), 228 (K) (16), 230 (K) (5), 233 (K) (5), 234 (K) (16), 242 (K) (16), 243 (K) (7), 249 (K) (16), s.n. (K) (5); Robert, G. 16608 (K) (6a); Robertson, S. A. 108 (K) (4), 676 (K) (11), 4121 (K) (20); Robertson, V. C. 5 (K) (11), 31 (K) (16); Roberty 16608 (K) (21), 16621 (K) (23); Robin, R. J. 3839 (K) (13), 4339 (K) (13), 4513 (K) (13); Robins, S. 902 (K) (13); Robinson, E. A. 637 (K) (23), 1214 (K) (3), 1847 (K) (6b), 1961 (K) (10), 2176 (K) (13), 3330 (K)

(6b), 4024 (K) (6b), 6174 (K) (10), 6403 (K) (11), 6753 (K) (6b), 6897 (K) (13); Robson, N. K. B. 1083 (K) (17), 1094 (BM, K) (3), 1169 (BM) (10), 1487 (K) (13); Robson & Jackson 1179 (K) (10), 1199 (BM, K) (11); Robyn, L. 1971 (K) (17), 1980 (K) (13), 2216 (K) (16); Rodin, R. J. 9379 (K) (16); Roger 758 (K) (19); Rogers 1821 (GH) (16), 20943 (US) (10); Rogers, C. G. 560 (K) (10), 709 (K) (11), 740 (K) (11); Rogers, D. 96 (US) (4), 1493 (US) (6b); Rogers, F. A. 7023 (K) (13), 8215 (K) (13), 18297 (K) (6b); Rose, F. 10117 (K) (17), 10253 (K) (17); Ross, J. H. 1743 (K, MO) (10), 2240 (K) (6b); Rounce, N. V. 288 (K) (4), 355 (K) (11), 358 (K) (13), 405 (K) (13); Rowland 1893 (K) (23); Rudatis, F. 543 (US) (6b), 1829 (S) (6b); Rushworth, J. E. 1489 (K) (17); Russell, J. 41 (K) (6b); Russell, T. 4 (K) (17); Rutherford-Smith, R. O. 542 (K) (4), 565 (MO) (6b); Ruxton, J. P. 53 (K) (13), 67 (K) (11), 149 (K) (11), 152 (K) (5); Rwaburindore, P. K. 580 (MO) (6e), 817 (MO) (6e), 1813 (US) (11); Ryan, J. B. H40/33 (K) (11); Ryan, J. D. 28 (K) (16), 58 (K) (16); Ryder, E. F. 102 (K) (10); Ryding, O. 120 (UPS) (16); Ryding, O. et al. 1548 (K) (16), 1567 (K) (16).

Saadou, M. 1839 (K) (5); Safa, S. B. & L. J. Musselman 10/3/87 (ODU) (6e), 10/9/87 (ODU) (13g), 10/9/87 (ODU) (16), 10/9/87 (ODU) (24), 10/9/87 (ODU) (7), 10/10/87 (ODU) (13h), 10/10/87 (ODU) (5), 10/10/87 (ODU) (7), 10/12/87 (ODU) (5), 10/12/87 (ODU) (7), 10/13/87 (ODU) (22), 10/13/87 (ODU) (5), 10/13/87 (ODU) (7), 10/19/87 (ODU) (24), 10/21/87 (ODU) (6e), 10/21/87 (ODU) (7), 10/25/87 (ODU) (17), 10/25/87 (ODU) (22); Sallinho Botanical Exped. 3824 (K) (23); Salubeni, A. J. 278 (K) (13), 416 (K) (17), 424 (K) (11), 1330 (GH, K, MO) (14), 1341 (K) (14), 1453 (K) (17), 1539 (K) (14); Sanane, M. 980 (K) (6b), 1571 (K) (13); Sanders, J. R. 54 (K) (10), 62 (K) (4); Sanderson, J. 428 (K) (6b); Saunders, D. H. 7 (K) (16), 60 (K) (16), 62 (K) (16); Sawer, E. R. 13698 (K) (16), 13699 (K) (16); Saxer, A. 254 (K) (6d), 294 (K) (17), 308 (K) (16), 309 (K) (11), 398A (K) (16), 588 (K) (16); Schenpera, J. C. 1048 (K, MO) (6b); Schimper, M. W. 319 (K) (13); Schinz, H. 35 (K) (13), 42 (K) (16); Schlechter, R. 3814 (GH, K, MO) (6b), 3979 (GH, MO) (10), 4342 (K) (13), 4541 (GH) (4); Schlieben, H. J. 2108 (US) (14), 2281 (K, S) (27), 3874 (K) (11), 5791 (K) (13), 6021 (S) (27), 7662 (US) (10), 7668 (US) (6b); Scholes, J. S9 (K) (6e); Schuter, P. A. & A. Nuvunga 6644 (K) (6b); Schweinfurth, G. 179 (K) (11), 595 (K) (16), 1880 (US) (16), 1992 (S) (5), 2285 (K) (4); Schyff, H. 1897 (K) (11); Scott, H. 217 (K) (16); Semsei, S. R. 1720 (K) (27), A9/48 (K) (4); Seydel, R. 4491 (K, US) (13); Shannon, R. K. 88–39 (US) (5), 88–44 (US) (13); Shantz, H. L. 930 (K) (16); Shantz & Tanner 4196 (K) (13); Sherif, M. I. 3952 (K) (17); Shillito, E. M. 119 (K) (11); Shorland 179 (K) (16); Shuell 6785 (K) (19); Siame, W. 95 (K) (4), 112 (K) (11), 144 (K) (24), 144A (BM) (7), 164 (K) (13), 525 (MO) (6b); Sidey, J. L. 1287 (UPS) (6b), 1589 (MO) (6b); Sieber 2401 (GH, K) (4); Sighton, F. C. 1298 (BM) (22), 2042 (BM) (22); Sillitoe, F. 457 (K) (17); Simfsuz, N. D. 2166 (K) (16); Simon, B. K. & G. Williamson 1403 (K) (6b); Simpson, N. D. 7302 (K) (17), 7406 (K) (16), 7606 (K) (16); Simson, H. E. 348 (K) (16); Skarpe, C. 52 (K) (13); Smith, L. C. 10/92 (K) (20); Smith, P. A. 26 (K) (13), 390 (K, MO) (6b), 830 (K) (13), 845 (K) (4), 887 (K) (13), 1369 (MO) (10), 1376 (K) (13), 1970 (K, MO) (6b), 2259 (K, MO) (10), 3114 (K) (13); Smith, P. A. et al. 5872 (K) (13); Smith, S. H. 22673 (K) (11); Smuts, J. C. & M. C. Gillett 3399 (K) (6b), 4127 (K) (6b); Snowden, J. D. 1160 (K) (16); Someren, V. 250 (K) (17), 7651 (K) (13); Sommerville, S. S. 726A (BM) (11); Sousa, A. F. 445 (K) (6b), 555 (K) (4); Soyaux, H. 189 (K) (4); Speke & Grant 572 (K) (16); Spinagi, C. A. 361 (K) (17); Stee, J. V. 3163 (K) (4); Stewart, D. R. M. 327 (K) (11); Stewart, M. M. 1/1911 (K) (6b); Steyagi, M. F. s.n. (US) (16); Stirton, C. H. 401 (K, MO) (6b), 559 (K) (6b), 1458 (K) (10), 1725 (K) (13); Stocks, J. 55 (K) (27); Stolz, A. 213 (GH, US) (11), 540 (K) (17), 753 (K) (13), 1304 (BM, K, GH, MO) (14), 2494 (GH, MO) (10), 2500 (GH) (19); Strey, R.G. 6187 (K) (6b), 9297 (K) (6b), 9987 (K) (6b); Suabad, M. M. 6994 (MO) (23); Sutherland, D. 1856 (K) (10); Sutherland, J. 381 (MO) (13a); Swynnerton, C. F. 1413 (K) (13), 1451 (K) (27), 1951 (K) (27); Symes, Y.E. 112 (K) (11).

T.C.D. Biol. Exped. 210 (BM) (13); Tadesse, F. M. & K. Vollesen 3073 (K) (20); Tadesse, M. T. & G. Y. Yagnew 1594 (K) (16), 1600 (K) (16); Talbot, P. A. 308 (BM) (11), 310 (K) (16), 312 (K) (7), 313 (BM) (6d), 324 (BM, K) (23), 1920 (BM) (24); Tanner, R. 89 (K) (27), 129 (K) (4), 747 (K) (16), 810 (K) (4), 1690 (K) (10), 1979 (K) (4), 2197 (K) (27), 2658 (K) (27), 2850 (K) (27), 2879 (K) (11), 2904 (K) (11), 2947 (K) (4), 2954 (K) (22), 3018 (K) (4), 3996 (K) (13), 4142 (K) (16), 4290 (K) (16), 4461 (K) (10), 5108 (K) (6a), 5274 (K) (16), 5655 (K) (6a), S110 (K) (6a); Tapscott, S. 2548 (K) (10); Taylor, W. W. 1885 (BM) (11); Taylor, T. W. 1 (K) (17); Teague, A. J. 33 (K) (13); Teixerira, B. 303 (BM) (6e); Terry, P. J. 1879 (K) (5), 1942 (K) (5), 3104 (K) (5), 3135 (K) (5), 3173 (K) (5), 3384 (K, ODU) (20), s.n. (K) (16); Thomas 4429 (K) (6e); Thomas, A. S. 183 (K) (17), 1031 (K) (16), 1532 (K) (16), 2015 (K) (11), 2015 (K) (11), 2893 (K) (11), 3458 (K) (13), 3554 (K) (13), D138 (K) (6f); Thomas, D. 2452 (MO) (6d); Thomas, N. W. 155 (K) (6f), 603 (K) (6f), 3164 (K) (23), 3208 (K) (23), 5829 (K) (23), 6665 (K) (23); Thompson 10 (K) (23); Thomson, B. s.n. (K) (13); Thorneiop, G. 2206 (K) (11); Thorold, C. A. 209 (K) (6e), 251 (BM) (24); Thulin, M. & B. A. Mahamed 7088 (K) (13); Thulin, M. & B. Mhoro 488 (K) (4), 3140 (K) (4); Timberlake, J. R. 459 (K) (13); Tolken, H. & D. S. Hardy 919 (K) (13); Toms, K. E. 5 (K) (4); Toul Botanical Exped. 1464 (K) (23), 1699 (K) (23); Towa, E. 8166 (K) (19); Townsend, C. C. 170 (K) (17), 2162 (K) (13); Troprell, C. G. 2050 (K) (4); Troupin, G. 621 (K) (11); Tutin, C. E. 162 (K) (6a); Tweedie 638 (K) (4), 2448 (K) (13), 3314 (K) (13), 3360 (K) (13), s.n. (K) (4); Twuner, A. 6730 (K) (13); Tyson, W. 894 (GH) (6b), 1222 (GH) (4), 1368 (MO) (10), 1504 (GH) (4).

Vallance, G. B. 699 (K) (4), 700 (K) (4); Vallah 65 (K) (1); Vaughan, J. H. 402 (K) (11), 1613 (K) (11), 1614 (K) (17), 1664 (K) (11), 2328 (BM) (27); Verboom, W. C. 1039 (K) (13); Verdcourt, B. 581 (K) (13), 3330 (K) (6e), 3390 (K) (17), 3690 (K) (20); Vigne, C. 4359 (US) (6e); Visser, J. H. 248 (K) (10), 315 (K) (6b), 316 (K) (6b), 2528 (K) (13); Vollesen, K. 3311 (K, UPS) (24); Vrgine, G. 3355 (K) (6e); Vuuren, D. A. & W. Giess 1132 (K) (13).

Waitland 838 (K) (6e); Walich, Z. 1278 (K) (10); Wallace, G. B. 713 (K) (27); Walter, E. G. 10 (K) (13); Wanntop, H. E. 515 (K) (13), 760 (K) (13), 814 (K) (13); Welch, J. R. 174 (K) (16); Wellby, M. S. 1901 (K) (16); Wellman, J. C. 1769 (K) (4); Welwitsch, A. P. 779 (K) (13), 5851 (K) (10); Werdermann & Oberdieck 2236a (US) (4); Wham, J. R. D. 26/2/24 (K) (11); Whellar, J. A. 1188 (K) (24), 1214 (K) (11); White, F. 2048 (K) (13), 6213 (K) (10), 7574 (K) (11); White, M. 142 (K) (13); Whiteside, J. O. 107793 (K) (11), 130476 (K) (11); Whyte, A. 1898 (K) (16), 1898 (K) (17), 1902 (K) (13), 1912 (K) (13); Wickens, G. E. 309 (K) (16), 536 (K) (13), 568 (K) (16), 587 (K) (13), 628 (K) (16), 1562 (K) (23), 2063 (K) (24), 2099 (K) (17), 2241 (K) (17), 2306 (K) (24), 2332 (K)

(24), 2345 (K) (24), 2492 (K) (16), 2807 (K) (23), 3159 (K) (13), Wiehe, P. O. 28294 (K) (4), N/118 (K) (11), N/426 (K) (4); Wild, H. 599 (K) (11), 1631 (K) (13), 4315 (K) (10), 5718 (K) (17); William, M. 5975 (K) (13); Willemse, R. H. 30 (K) (16); Williams, G. R. 220 (K) (11); Williams, J. G. 5115 (K) (13), 12546 (K) (13), B5113 (K) (4); Williams, R. 545 (K) (16); Williams, R. H. 32 (K) (4); Williams, T. L. 534 (K) (6e); Williamson, D. J. 90 (K) (4); Wilson 349 (K) (16); Wilson, J. 373 (K) (13), 880 (K) (11); Winter, B. D. 2540 (K) (6b), 3372 (K) (13), 3995 (K) (6b), 4428 (K) (6b); Winter, B. D. & O. A. Leistner 5368 (K) (13); Winter, B. D. & W. Marais 4478 (K) (6b), 4479 (K) (13), 4567 (K) (10), 4731 (K) (13), 4913 (K) (6b); Winter, B. D. & H. J. Wise 4452 (K) (13); Wit et al. 166671 (K) (11); Wittle, D. 314 (K) (16); Wood, G. H. S. 726 (K) (6e), 805 (K) (13); Wood, J. M. 4/1908 (US) (4), 16 (K) (4), 20 (US) (4), 113 (K) (6b), 394 (K) (10), 440 (K) (11), 911 (US) (6b), 4276 (K) (13), 4934 (MO) (6b), 7252 (US) (6b), 7500 (US) (11), 9212 (GH) (6b), 10495 (MO) (6b), 10873 (US) (4), 10887 (US) (6b), 92715 (MO) (10); Woolhouse, H. W. 51/61/22 (K) (17); Worsdell, W. O. 12/1909 (K) (6b); Wright, J. M. 302 (K) (13); Wyld, J. W. 225 (K) (4), 725 (BM) (6a); Wylie, F. 1895 (K) (6b).

Yeafes, M. 88 (K) (16); Yong, J. D. 184 (K) (11); Young, J. 185 (K) (6f); Young, R. G. 1261 (ODU) (2), 1300 (MO) (1), 1347 (BM) (2), 1365 (MO) (2).

Zimmer, F. 102 (K) (27); Zunguze, D. & X. Boan 49 (K) (11).

PHYLOGENY AND HISTORICAL BIOGEOGRAPHY OF LAURACEAE: EVIDENCE FROM THE CHLOROPLAST AND NUCLEAR GENOMES[1]

André S. Chanderbali,[2,3] Henk van der Werff,[3] and Susanne S. Renner[2,3]

ABSTRACT

Phylogenetic relationships among 122 species of Lauraceae representing 44 of the 55 currently recognized genera are inferred from sequence variation in the chloroplast and nuclear genomes. The *trn*L-*trn*F, *trn*T-*trn*L, *psb*A-*trn*H, and *rpl*16 regions of cpDNA, and the 5′ end of 26S rDNA resolved major lineages, while the ITS/5.8S region of rDNA resolved a large terminal clade. The phylogenetic estimate is used to assess morphology-based views of relationships and, with a temporal dimension added, to reconstruct the biogeographic history of the family. Results suggest Lauraceae radiated when trans-Tethyean migration was relatively easy, and basal lineages are established on either Gondwanan or Laurasian terrains by the Late Cretaceous. Most genera with Gondwanan histories place in Cryptocaryeae, but a small group of South American genera, the *Chlorocardium–Mezilaurus* clade, represent a separate Gondwanan lineage. *Caryodaphnopsis* and *Neocinnamomum* may be the only extant representatives of the ancient Lauraceae flora documented in Mid- to Late Cretaceous Laurasian strata. Remaining genera place in a terminal Perseeae–Laureae clade that radiated in Early Eocene Laurasia. Therein, non-cupulate genera associate as the *Persea* group, and cupuliferous genera sort to Laureae of most classifications or Cinnamomeae sensu Kostermans. Laureae are Laurasian relicts in Asia. The *Persea* group and *Cinnamomum* group (of Cinnamomeae) show tropical amphi-Pacific disjunctions here credited to disruption of boreotropical ranges by Eocene–Oligocene climatic cooling. The *Ocotea* complex accommodates remaining Cinnamomeae and shows a trans-Atlantic disjunction possibly derived from a Madrean-Tethyan ancestral distribution. These findings support Laurasian ancestry for most extant Lauraceae, with their considerable neotropical representation primarily derived from Early Miocene radiation of the *Ocotea* complex upon reaching South America.

Key words: biogeography, boreotropical, chloroplast DNA, Gondwana, Lauraceae, Laurasia, Madrean-Tethyan, molecular clock, phylogeny, ribosomal DNA.

Lauraceae form a large family of woody plants (except the herbaceous parasite *Cassytha*), with about 50 genera and 2500 to 3000 species distributed throughout tropical to subtropical latitudes. They are among the more speciose basal angiosperm families and have a fossil record that reaches back to the Mid-Cretaceous (Drinnan et al., 1990; Eklund & Kvaček, 1998). Current taxonomic diversity is centered in tropical America and Australasia, and although poorly represented in continental Africa, Lauraceae flourish in Madagascar. In the American tropics they list among the most prominent components of lowland forests and are frequently dominant elements in montane vegetation (Gentry, 1988).

Given their antiquity, widespread distribution, and ecological prominence, Lauraceae provide a model system for investigating angiosperm biogeography. Moreover, the three tribes recognized by van der Werff and Richter (1996) suggest that major divisions in the family draw along geographic lines. Laureae include three genera with North American–Asian disjunctions (*Litsea, Lindera, Sassafras*), Mediterranean *Laurus*, and Asian endemics (e.g.,

[1] The authors thank anonymous reviewers for insightful comments; K. Ueda for eight unpublished *trn*L-*trn*F sequences; J. Rohwer for six unpublished ITS sequences; M. Zanis for five at the time unpublished ITS/5.8S/26S sequences; Y-L. Qiu for providing three DNA aliquots; the Harvard Herbaria for providing leaf material of *Eusideroxylon*; Andrea Schwarzbach for guidance in molecular techniques; and innumerable colleagues who helped to collect material of Lauraceae for this study. This study was undertaken as part of ASC's doctoral dissertation research at the University of Missouri-St. Louis, and was supported by grants from the Mallinckrodt Foundation administered by the International Center for Tropical Ecology, University of Missouri-St. Louis, and from the Smithsonian Institution's Biological Diversity of the Guianas Program to ASC; National Geographic Society grants 4631-91, 5635-96, and 6571-99 to HW; and grants from the University of Missouri system and University of Missouri–St. Louis to SSR. This article is Number 45 in the Smithsonian Institution's Biological Diversity of the Guianas Publication series.

[2] Department of Biology, University of Missouri–St.Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121-4499, U.S.A.

[3] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, U.S.A.

Actinodaphne and *Neolitsea*). Cryptocaryeae include pantropical *Beilschmiedia* and *Cryptocarya*, and other predominantly Southern Hemispheric genera with narrower ranges (e.g., *Endiandra* and *Potameia*). Perseeae are centered in the Neotropics with three widespread genera. *Cinnamomum* is disjunct between the American and Asian (sub)tropics; *Persea* (including *Machilus*) ranges throughout the Neotropics (into the southeastern U.S.), the Canary Islands, and Asia; and *Ocotea* has about 300 neotropical species, one in Macaronesia, a few in Africa, and about 30 more in Madagascar. Apart from a few Asian genera (e.g., *Dehaasia* and *Phoebe*) considered closely related to *Persea*, all other genera of Perseeae are endemic to the Neotropics (e.g., *Aiouea*, *Aniba*, *Endlicheria*, *Licaria*, *Nectandra*, *Pleurothyrium*, and *Rhodostemonodaphne*). The distributions of Laureae and Cryptocaryeae are consistent with Laurasian and Gondwanan histories, respectively, but that of Perseeae is ambiguous in this regard. The trans-Atlantic distribution of *Ocotea* suggests West Gondwanan history, but the tropical amphi-Pacific distributions of *Cinnamomum* and *Persea* suggest Laurasian affinities. Whether or not these ambiguities are artifacts of tribal and/or generic circumscription is unclear.

The systematics of Lauraceae is unsettled. Laureae are recognized in most prior classifications, but other tribal concepts are not widely accepted. Van der Werff and Richter's (1996) Cryptocaryeae and Perseeae are revised concepts supported by Richter's (1981) study of wood and bark anatomy. Some aspects of Cryptocaryeae gain support from embryology (Heo et al., 1998), but characters for further subdivision of the family were not found. A cladistic analysis of molecular data (Rohwer, 2000) also provided support for Cryptocaryeae and united Perseeae and Laureae in a well-supported but unresolved clade. This Perseeae–Laureae clade accommodates most of the family, and its distribution implies a disjunction between the American and Asian tropics with a minor African presence. Such distributions can be credited to extinction of Gondwanan lineages in Africa (e.g., Raven & Axelrod, 1974), but Laurasian ancestry followed by radiation in tropical Asia and America was favored by Rohwer (2000). Tropical Asia is acknowledged as a harbor for Laurasian relicts (e.g., Wolfe, 1975), but Rohwer's hypothesis that the approximately 800 neotropical species of Perseeae are derived from Laurasian immigrants contrasts with the Gondwanan origin credited to most major neotropical plant groups (e.g., Raven & Axelrod, 1974; Gentry, 1982; references in Goldblatt, 1993; Burnham & Graham, 1999). Further, Raven and Axelrod (1974: 563) allowed that *Persea* was an "old Laurasian genus" but suggested that subgenus *Eriodaphne*, like all other members of the family, arrived in South America via Africa. Alternatively, Taylor (1988) suggested that the fossil flower *Androglandula tennessensis* Taylor, from the Eocene of North America, is comparable to *Cinnamomum*, *Ocotea*, and *Nectandra*, indicating a boreotropical history for this group of genera, with subsequent migration to South America. Raven and Axelrod (1974) and Taylor (1988) allowed that although differing in the direction of migration, the high level of species diversity and generic endemism require a Late Cretaceous–Early Tertiary arrival in South America. In contrast, Rohwer and Kubitzki (1993) preferred a more recent arrival of this group, possibly as late as the Pliocene closure of the Panamanian Isthmus, followed by rapid radiation in South America.

These alternative views are here assessed by adding a temporal dimension to a phylogenetic estimate for Lauraceae reconstructed with molecular characters retrieved from both chloroplast and nuclear genomes.

Materials and Methods

Taxon and Molecular Sampling

A total of 131 species, 122 representing 44 of the 55 currently recognized genera of Lauraceae, and 9 representing 3 outgroup families (Gomortegaceae, Hernandiaceae, and Monimiaceae) were included in this study. Seventy-seven in-group species were included in a broad-scale study based on sequence variation in the *trn*L-*trn*F and *psb*A-*trn*H intergenic spacers of cpDNA. Guided by the findings of this molecular and taxon sampling a two-tiered approach was adopted to obtain better representation and phylogenetic resolution at the generic level. Thus a subset of the species representing basal lineages in Lauraceae was also sequenced for the *trn*T-*trn*L spacer, and the *rpl*16 intron of cpDNA, as well as the 5′ end of 26S rDNA, while 94 species representing Perseeae, Laureae, and their sister group were sampled for the ITS/5.8S region of nrDNA. Table 1 provides GenBank information for all accessions.

DNA Extraction, PCR Amplification, and Sequencing

Total DNA was obtained from silica-gel-dried, herbarium, or fresh leaves using Dneasy (QIAGEN) extraction kits. PCR amplification of chloroplast loci was conducted using standard protocols (e.g., White et al., 1990). For nuclear markers, 10%

Table 1. Sources of plant material. [1]Previously submitted to Genbank. Contributed by: [2]J. Rohwer, Univ. Hamburg; [3]K. Ueda, Univ. Osaka.; and [4]M. Zanis, Washington State Univ.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Lauraceae								
Actinodaphne sesquipedalis Meisn.	Malaysia, Kuala Lumpur	*Saw Leng Guan s.n.* (KEP)	AF268695	AF268787	—	AF268239	—	AF272247
Adenodaphne uniflora (Guill.) Kosterm.	New Caledonia, Province du Sud	*van der Werff 15895* (MO)	—	—	—	—	—	AF272248
Aiouea costaricensis (Mez) Kosterm.	Costa Rica, Heredia	*Grayum 8241* (HBG) (J. Rohwer sequence)	—	—	—	—	—	AF272249[2]
Aiouea dubia (HBK) Mez	Ecuador, Loja	*Madsen 75433* (AAU) (J. Rohwer sequence)	—	—	—	—	—	AF272250[2]
Aiouea guianensis Aubl.	Guyana, Demerara, Timerhi	*Taylor 12085* (MO)	AF268696	AF268780	—	—	—	AF272251
Alseodaphne semecarpifolia Nees	Sri Lanka, Central Prov, Kandy	*Malcomber 2753* (MO)	AF268697	AF268799	—	—	—	AF272252
Anaueria brasiliensis Kosterm.	Peru, Loreto, Iquitos	*Vásquez 25228* (MO)	AF268698	AF268800	AF268840	AF268247	—	AF272253
Aniba cinnamomiflora C. K. Allen	Venezuela, Trujillo, Boconó	*Cuello 955* (MO)	AF268700	AF268770	AF268823	—	—	AF272254
Aniba excelsa Kosterm.	Guyana, Demerara, Mabura Hill	*Chanderbali 226* (MO)	—	—	—	—	—	AF272255
Aniba hypoglauca Sandwith	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 165* (MO)	AF268699	AF268771	AF268822	—	—	—
Aniba panurensis (Meisn.) Mez	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 248* (MO)	—	—	—	—	—	AF272256
Apollonias barbujana (Cav.) Bornm.	Canary Islands, Tenerife	*Bramwell 628* (MO)	—	—	—	—	—	AF272257
Aspidostemon sp.	Madagascar, Toliara	*van der Werff 12737* (MO)	AF268701	AF268819	AF268843	AF268251	—	—
Beilschmiedia brenesii C. K. Allen	Costa Rica, Puntarenas	*Yasuda 1310* (MO)	AF268702	AF268809	—	—	AF262004[1]	—
Beilschmiedia madagascariensis Kosterm.	Madagascar, Toliara	*Lowry 5015* (MO)	—	AF268810	—	—	—	—
Beilschmiedia ovalis (Blake) C. K. Allen	Costa Rica, San Jose	*Yasuda 1301* (MO)	AF268703	AF268811	—	—	AF270472	—
Beilschmiedia sary Kosterm.	Madagascar, Toamasina	*van der Werff 12800* (MO)	—	AF268812	—	—	—	—

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Beilschmiedia tilaranensis Nishida	Costa Rica, Guanacaste	*Yasuda 1313* (MO)	AF129014[1]	AF129045[1]	AF129015[1]	AF127265[1]	—	—
Beilschmiedia velutina (Kosterm.) Kosterm.	Madagascar, Antsiranana	*Georges 361* (MO)	AF268704	AF268813	—	—	—	—
Caryodaphnopsis bilocellata van der Werff	Vietnam, Ninh Binh	*van der Werff 14195* (MO)	AF23032[1]	AF261995[1]	AF233603[1]	AF232743[1]	AF262005[1]	—
Caryodaphnopsis tomentosa van der Werff	Peru, Loreto, Iquitos	*Vásquez 25239* (MO)	AF268705	AF268807	AF268842	AF268248	—	—
Cassytha filiformis L.	Guyana, Rupununi, Karanambo	*Chanderbali 205* (MO)	AF232034[1]	AF261996[1]	AF233605[1]	AF232744[1]	AF262006[1]	—
Cassytha pubescens R. Br.	Australia, Victoria, Geelong	*Foreman 1913* (MEL)	AF232033[1]	—	AF233604[1]	—	AF262007[1]	—
Chlorocardium rodiei (R. H. Schomb.) Rohwer, H. G. Richt. & van der Werff	Guyana, Demerara, Maburra Hill	*Chanderbali 246* (MO)	AF268706	AF268802	—	AF268245	—	AF272258
Chlorocardium venenosum (Kosterm. & Pinkley.) Rohwer, H. G. Richt. & van der Werff	Peru, Loreto, Iquitos	*Vásquez 25236* (MO)	AF268707	AF268801	AF268840	—	—	AF272259
Cinnamomum camphora (L.) Presl.	Missouri BG	*Chanderbali 322* (MO)	AF129020[3]	AF129048[1]	AF129021[1]	AF129019[1]	—	AF272260
Cinnamomum chavarrinum (Hammel) Kosterm.	Costa Rica	*Gomez-Laurito s.n.* (J. Rohwer sequence)	—	—	—	—	—	AF272261[2]
Cinnamomum cinnamomifolium (HBK) Kosterm.	Ecuador	*Thomsen 8942* (AAU) (J. Rohwer sequence)	—	—	—	—	—	AF272262[2]
Cinnamomum japonicum Siebold	Japan, Honshu, Kyoto	*Yasuda 1351* (MO)	AF268708[3]	AF268782	—	—	—	AF272263
Cinnamomum oleifolium (Mez) Kosterm.	Brazil, Minas Gerais	*Lorea-Hernandez 5582* (MO)	—	—	—	—	—	AF272264
Cinnamomum quadrangulum (Meisn.) Kosterm.	Brazil, Minas Gerais	*Lorea-Hernandez 5585* (MO)	—	AF268781	—	—	—	AF272265
Cinnamomum sp.	Vietnam, Lao Cai	*van der Werff 14396* (MO)	—	AF268783	—	—	AF270473	AF272266
Cinnamomum verum Presl.	India, Kumbhave, Dapoli	*Godbole 45108* (MO)	AF268709	AF268784	—	—	—	AF272267
Cryptocarya chinensis (Hance) Hemsl.	Asia	K. Ueda sequence	AF268710[3]	—	—	—	—	—

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Cryptocarya rhodosperma Hyland	Australia, Queensland	*Gray 7556* (QRS)	AF268711	AF268817	—	—	AF270474	—
Cryptocarya sclerophylla Hyland	Brisbane City Botanic Garden	*Schwarzbach s.n.* (voucher destroyed)	AF268712	AF268818	—	—	—	—
Cryptocarya thouvenotii (Danguy) Kosterm.	Madagascar, Toliara	*van der Werff 12723* (MO)	AF232035[1]	AF261997[1]	AF233606[1]	AF232745[1]	AF262008[1]	—
Dehaasia incrassata (Jack) Kosterm.	Philippines, Palawan, Taytay	*Soejarto 7693* (MO)	—	—	—	—	—	AF272268
Dicypellium caryophyllaceum (Mart.) Nees	Brazil, Para	*Pires 16756* (HBG)	—	—	—	—	—	AF272269
Dicypellium manausense W. Rodr.	Brazil, Amazonas, Manaus	*Assunção 749* (INPA)	AF268713	AF268775	AF268830	—	—	AF272270
Endiandra microneura C. White	Australia, Queensland	*Schulman s.n.* (QRS)	AF268714	AF268814	—	—	AF270475	—
Endlicheria chalisea Chanderbali	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 252* (MO)	AF268715	AF268756	AF268829	—	—	AF272271
Endlicheria citriodora van der Werff	Peru, Loreto, Iquitos	*Vasquez 25231* (MO)	AF268716	AF268757	—	—	—	AF272272
Endlicheria punctulata (Mez) C. K. Allen	French Guiana, Tumac Humac	*de Granville 1448* (MO)	—	—	—	—	—	AF272273
Endlicheria reflectens (Nees) Mez	Guyana, Essequibo, Rupununi	*Chanderbali 208* (MO)	AF268717	AF268758	AF268828	—	—	AF272274
Gen. & sp. nov. aff. *Mezilaurus*	Peru, Loreto, Iquitos	*Vásquez 25230* (MO)	AF268719	AF268803	AF268838	—	AF270483	—
Eusideroxylon zwageri Teijsmann & Binnendijk	Borneo, Kalimantan	*Laman 1275* (HUH)	AF268718	AF268820	—	AF268252	—	—
Hypodaphnis zenkeri (Engl.) Stapf	Gabon, Ogooue-Ivindo, Lopé-Okanda	*McPherson 16184* (MO)	AF232036[1]	AF261998[1]	AF233607[1]	AF232746[1]	AF262009[1]	—
Iteadaphne sp.	Vietnam, Lao Cai	*van der Werff 14360* (MO)	AF268720	AF268786	AF268834	AF268241	—	AF272275
Kubitzkia mezii (Kosterm.) van der Werff	Guyana, Essequibo, Iwokrama	*Chanderbali 249* (MO)	AF268721	AF268772	—	—	AF270476	AF272276
Laurus nobilis L.	Missouri BG	*Chanderbali 327* (MO)	AF268722	AF268785	AF268835	—	—	AF272278
Licaria cannella (Meisn.) Kosterm.	Guyana, Demerara, Mabura Hill	*Chanderbali 234* (MO)	AF268723	AF268773	—	—	—	AF272280
Licaria guianensis Aubl.	Brazil, Amazonas, Manaus	*Vicentini 1238* (MO)	—	—	—	—	—	AF272281

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Licaria martiniana (Mez) Kosterm.	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 264* (MO)	—	—	—	—	—	AF272279
Licaria triandra (Sw.) Kosterm.	Fairchild Tropical Garden, Fl., U.S.A.	*Qiu 90019* (NCU)	AF268724	AF268774	—	—	—	AF272282
Lindera benzoin (L.) Blume	Missouri BG	*Chanderbali 324* (MO)	AF268725[3]	AF268788	AF268833	—	—	AF272283
Lindera erythrocarpa Makino	Japan, Honshu, Kyoto	*Yasuda 1353* (MO)	AF268726[3]	—	—	—	—	AF272284
Lindera umbellata Thunb.	Japan, Honshu, Kyoto	*Yasuda 1354* (MO)	AF268727[3]	AF268789	—		—	AF272285
Litsea coreana Leveille	Japan, Honshu, Kyoto	*Yasuda 1356* (MO)	AF268728[3]	AF268791	—	—	AF262010[1]	AF272286
cf. *Litsea elongata* (Nees) Benth. & Hook. f. (Distributed to MO as *Phoebe forrestii* W. W. Sm.)	China, Yunnan, Kunming BG	*Hyland 14912* (MO)	—	—	—	—	—	AF272277
Litsea glaucescens HBK	Mexico, Guerrero, Chichihualco	*Lorea-Hernandez 5496* (MO)	AF129035[1]	AF129063[1]	AF129036[1]	AF127266[1]	—	—
Mezilaurus triunca van der Werff	Peru, Amazonas, Iquitos	*Vásquez 25227* (MO)	AF268729	AF268804	AF268837	AF268246	—	AF272287
Mocinnodaphne cinnamomoidea Lorea-Hern.	Mexico, Guerrero, El Molste	*Lorea-Hernandez 5536* (MO)	—	—	—	—	—	AF272288
Nectandra amazonum Nees	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 217* (MO)	—	—	—	—	—	AF272289
Nectandra coriacea (Sw.) Griseb.	U.S.A. Florida, Monroe County	*Prinzie 125* (MO)	—	—	—	—	—	AF272290
Nectandra cuspidata Nees & Mart.	Guyana, Essequibo, Kamarang	*Chanderbali 279* (MO)	—	—	—	—	—	AF272291
Nectandra membranacea (Sw.) Griseb.	Brazil, Sao Paulo, Serra do Mar	*Lorea-Hernandez 5596* (MO)	AF268730	AF268767	AF268825	AF268244	—	—
Nectandra psammophila Nees & C. Mart.	Brazil, Sao Paulo, Serra do Mar	*Lorea-Hernandez 5595* (MO)	—	—	—	—	—	AF272292
Nectandra purpurea (Ruiz & Pav.) Mez	Peru, Cajamarca, San Ignacio	*Campos et al. 3165* (MO)	—	—	—	—	—	AF272293
Nectandra salicifolia (HBK) Nees	Costa Rica	*Gomez-Laurito s.n.* (J. Rohwer sequence)	—	—	—	—	—	AF272294[2]
Nectandra turbacensis (HBK) Nees	Puerto Rico, Rio Grande, El Verde	*Taylor 11746* (MO)	AF012400[1]	AF268768	AF268826	—	—	AF272295

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Neocinnamomum mekongense (Hand.-Mazz) Kosterm.	China, Yunnan	*Li Heng 8547* (MO)	AF268731	AF268806	AF268841	AF268249	AF270477	—
Neolitsea sericea (Blume) Koidz.	Japan, Honshu, Kyoto	*Yasuda 1355* (MO)	AF268732[3]	AF268792	—	AF268243	AF270478	AF272296
Ocotea botrantha Rohwer	U. C. Riverside	*Scora 99–1* (UCR)	AF268733	AF268776	—	—	—	AF272297
Ocotea bullata (Burchell) E. Mey.	South Africa, Natal, Skyline Arboretum	*Abbot 6208* (MO)	AF268734	AF267778	—	—	—	AF272298
Ocotea ceanothifolia (Nees) Mez	Guyana, Demerara, Mabura Hill	*Chanderbali 244* (MO)	—	—	—	—	—	AF272299
Ocotea foetens (Aiton) Baill.	Madeira, Porto Moniz	*Maas 8642* (MO)	AF268735	—	—	—	—	AF272300
Ocotea grayi van der Werff	Madagascar, Toliara	*van der Werff 12732* (MO)	AF268736	—	—	AF232747[1]	AF270479	AF272301
Ocotea guianensis Aubl.	Guyana, Demerara, Mabura Hill	*Chanderbali 232* (MO)	AF268737	AF268762	—	—	—	AF272302
Ocotea helicterifolia (Meisn.) Hemsl.	Mexico, Oaxaca, Miahuatlan	*Torres 11911* (MO)	—	—	—	—	—	AF272303
Ocotea heydeana (Mez & Donn. Sm.) Bernardi	Honduras, Yoro, Pico Pijol	*Evans 1760* (MO)	—	—	—	—	—	AF272304
Ocotea ikonyokpe van der Werff	Cameroon, SW Prov., Rumpi Hills	*Thomas 10456* (MO)	—	—	—	—	—	AF272305
Ocotea insularis (Meisn.) Mez	Costa Rica, Puntarenas, Coco Is	*Rojas 3682* (MO)	—	—	—	—	—	AF272306
Ocotea leucoxylon (Sw.) Laness.	Puerto Rico, San Jose	*Taylor 11733* (MO)	AF12399[1]	AF268763	—	—	—	—
Ocotea malcomberi van der Werff	Madagascar, Toliara	*van der Werff 12756* (MO)	—	AF268779	—	—	—	AF272307
Ocotea nigra Benoist	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 162* (MO)	—	—	—	—	—	AF272308
Ocotea odorifera (Vell.) Rohwer	Brazil, Minas Gerais, Parque Ecol. CEMIG	*Lorea-Hernandez 5578* (MO)	AF268738	AF268762	—	—	—	AF272309
Ocotea pauciflora (Nees) Mez	Guyana, Demerara, Mabura Hill	*Chanderbali 219* (MO)	—	AF268764	—	—	—	AF272310
Ocotea percoriacea (Mez) Kosterm.	Brazil, Goias, Fazenda Galheiro	*Lorea-Hernandez 5584* (MO)	AF268739	—	—	—	—	AF272311
Ocotea pulchella Mart.	Brazil, Minas Gerais, Fazenda Caiera	*Lorea-Hernandez 5575* (MO)	AF268740	—	—	—	—	AF262312

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Ocotea quixos (Lam.) Kosterm.	Ecuador, Napo, Jatun Sacha	*Neill 9487* (MO)	AF232037[1]	AF261999[1]	AF233608[1]	—	AF262011[1]	AF272313
Ocotea rhynchophylla (Meisn.) Mez	Guyana, Demerara, Mabura Hill	*Chanderbali 220* (MO)	—	AF268766	—	—	—	AF272314
Ocotea schomburgkiana (Nees) Mez	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 286* (MO)	—	—	—	—	—	AF272315
Ocotea spixiana (Nees) Mez	Brazil, Minas Gerais, Parque Ecol. Tripui	*Lorea-Hernandez 5574* (MO)	—	—	—	—	—	AF272316
Ocotea tomentella Sandwith	Guyana, Essequibo, Kamarang	*Chanderbali 284* (MO)	AF268741	AF268765	—	—	—	AF272317
Ocotea tristis (Nees & Mart.) Mez	Brazil, Minas Gerais, Parque Ecol. CEMIG	*Lorea-Hernandez 5577* (MO)	AF268742	—	—	—	—	AF272318
Ocotea veraguensis (Meisn.) Mez	Nicaragua, Chontales	*Stevens 24177* (MO)	—	—	—	—	—	AF272319
Paraia bracteata Rohwer, H. G. Richt. & van der Werff	Brazil, Amazonas, Iquitos	*Vicentini 1288* (MO)	AF268743	—	—	—	—	AF272320
Parasassafras confertiflora (Meisn.) Long	China, Yunnan, Lishui Co.	*Li Heng 10030* (MO)	—	AF268790	—	AF268238	AF270480	AF272321
Persea americana Mill.	Missouri BG	*Chanderbali 323* (MO)	AF268744	AF268794	—	—	—	AF272322
Persea caerulea (Ruiz & Pav.) Mez	Peru, Amazonas	*van der Werff 14744* (MO)	—	AF268795	—	—	—	AF272323
Persea lingue (Ruiz & Pav.) Nees ex Kopp	Chile	*Greissl 640–99* (MJG)	—	AF268796	—	—	—	AF272324
Persea meridensis Kopp	Venezuela, Trujillo, Boconó	*Cuello 943* (MO)	AF268745	AF267797	AF268836	—	—	AF272325
Persea sp.	Vietnam, Vinh Phuc	*van der Werff 14071* (MO)	—	—	—	—	—	AF272326
Persea thunbergii Siebold & Zucc.	Missouri BG	*Chanderbali 328* (MO)	AF268746	AF268798	—	—	—	AF272327
Phoebe formosana (Hayata) Hayata	Bonn BG	*Rohwer 156* (MJG) (J. Rohwer sequence)	—	—	—	—	—	AF272328[2]
Pleurothyrium cinereum van der Werff	Peru, San Martin, Riojas	*van der Werff 15325* (MO)	AF268747	AF268769	—	—	—	AF272329
Pleurothyrium insigne van der Werff	Ecuador, Napo, Jatun Sacha	*Neill 9033* (MO)	—	—	—	—	—	AF272330

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Potameia micrantha van der Werff	Madagascar, Toamasina	*van der Werff 12777* (MO)	AF268749	AF268815	—	—	AF270481	—
Potameia microphylla Kosterm.	Madagascar, Fianarantsoa	*van der Werff 12655* (MO)	AF268750	AF268816	—	AF268250	—	—
Potoxylon melagangai (Symington) Kosterm.	Brunei, Tutong, Kampong Lamumin	*Hyland 14969* (MO)	AF268748	AF268821	—	—	—	—
Rhodostemonodaphne crenaticupula Madriñán	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 265* (MO)	AF268751	AF268759	—	—	—	AF272331
Rhodostemonodaphne praeclara (Sandwith) Madriñán	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 256* (MO)	AF268752	AF268760	AF268827	—	—	AF272332
Rhodostemonodaphne recurva van der Werff	Brazil, Amazonas, Manaus	*Vicentini 653* (MO)	—	—	—	—	—	AF272333
Rhodostemonodaphne scandens Madriñán	Guyana, Essequibo, Iwokrama Reserve	*Chanderbali 271* (MO)	—	—	—	—	—	AF272334
Sassafras albidum (Nutt.) Nees	Missouri BG	*Chanderbali 325* (MO)	AF268753	AF268793	AF268832	AF268242	AF264140[4]	AF272335
Sassafras tzumu (Hemsl.) Hemsl	China, Hunan, Xining Co.	*Luo Lin-bo 1242* (MO)	—	—	—	—	—	AF272336
Sextonia pubescens van der Werff	Peru, Loreto, Iquitos	*Vásquez 25229* (MO)	AF232038[1]	AF262000[1]	AF233609[1]	AF232748[1]	AF262012[1]	AF268808
Sextonia rubra (Mez) van der Werff	Brazil, Amazonas, Manaus	*Nascimento 574* (MO)	AF268754	AF268805	—	—	AF270482	—
Umbellularia californica (Hook. & Arn.) Nutt.	Missouri BG	*Chanderbali 326* (MO)	AF268755	AF268777	—	—	—	AF272337
Urbanodendron bahiense (Meisn.) Rohwer	Brazil, Rio de Janeiro	*Martinelli 10019* (MO)	—	—	—	—	—	AF272338
Urbanodendron verrucosum (Nees) Mez	Brazil, Minas Gerais, Faz. Macedônea	*Braga s.n.* (BHCB) *19385* (MO)	—	—	—	—	—	AF272339
Gomortegaceae								
Gomortega nitida Ruiz and Pav.	Chile	*Rodriguez 3070* (CONC)	AF012404[1]	AF129053[1]	—	AF127260[1]	AF262014[1]	—
Hernandiaceae								
Gyrocarpus americanus Jacq.	Sri Lanka, Colombo BG	*Chase 317* (NCU)	AF012398[1]	AF129054[1]	AF129025[1]	AF127261[1]	AF262001[1]	—
Hernandia moerenhoutiana Guillem.	Australia, Brisbane, Mt. Cootha BG	not available	AF052198[1]	AF129055[1]	AF129026[1]	AF130310[1]	—	—

Table 1. Continued.

Taxon	Provenance	Voucher	Genbank accession numbers					
			*trn*L-*trn*F	*psb*A-*trn*H	*trn*T-*trn*L	*rpl*16	26S	ITS/5.8S
Illigera luzonensis (Presl) Merr.	Munich BG	not available	AF052199[1]	AF129057[1]	AF129030[1]	AF127264[1]	AF262002[1]	—
Sparrattenthelium wonotoboense Kosterm.	Munich BG	Munich BG 47/1311	AF053342[1]	AF129070[1]	AF129043[1]	AF127262[1]	—	—
Monimiaceae								
Hortonia floribunda Wight ex Arn.	Sri Lanka, Colombo BG	not available	AF040683[1]	AF129071[1]	AF129028[1]	AF129027[1]	AF264143[4]	—
Monimia ovalifolia Thouars	La Reunion	*Stasberg s.n.* (REU)	AF054896[1]	AF129065[1]	AF129038[1]	AF127269[1]	AF246144[4]	—
Palmeria scandens Muell.	Australia, New South Wales	*Bradford 878* (MO)	AF052200[1]	AF129067[1]	AF129040[1]	AF127270[1]	AF264142[4]	—
Peumus boldus Molina	Edinburgh BG	Edinburgh BG 19870707	AF012403[1]	AF129068[1]	AF129041[1]	AF127454[1]	AF264141[4]	—

DMSO was added and the PCR protocol described by Kuzoff et al. (1998) was followed. The *trn*L-*trn*F and *trn*T-*trn*L regions were amplified using primers designed by Taberlet et al. (1991). The *trn*L-*trn*F spacer sequences begin near the 5′ end of the spacer and include 138 bp of the 5′ end of the tRNA-Phe (*trn*F) gene. The spacer between tRNA-Leu (*trn*L) and tRNA-Thr (*trn*T) aligns readily with other lauralean *trn*T-*trn*L sequences produced by Renner (1999), but is difficult to align with the few available *trn*T-*trn*L sequences in GenBank, the most similar of which are *trn*T-*trn*L sequences from *Dioscorea* (48% similarity). The *psb*A-*trn*H spacer and *rpl*16 intron were amplified using primers of Sang et al. (1997) and Asmussen (1999), respectively. The *psb*A-*trn*H sequences obtained for Lauraceae include the entire spacer region and overlap by about 40 base pairs with the 3′ end of the *psb*A gene and 5′ end of the *trn*H gene of *Helianthus annuus* L. deposited in Genbank (X60428). The 5′ region that includes the first two expansion domains of 26S rDNA was amplified using a forward primer (D. Nickrent, pers. comm.) that anneals at ca. 70 nucleotide positions downstream from the 5′ end of the gene and the 641 R reverse primer of Kuzoff et al. (1998). To increase efficiency of PCR amplification of ITS, the few lauraceous sequences obtained using angiosperm-specific ITS A and ITS B primers (Blattner, 1999) were used to design a forward primer (5′-ACCACCACCGGCAACCA-3′) that anneals at about 10 bp downstream of the 3′ end of 18S. This primer (hereafter LAUR 1) appears to be specific to a large terminal clade in Lauraceae that includes the tribes Perseeae and Laureae and their sister group. In most cases it was possible to amplify the entire ITS region using LAUR 1 and ITS B, but for some poor-quality templates it was necessary to amplify the region in sections (ITS1 & ITS2) by combining the appropriate universal primers of White et al. (1990) with LAUR 1 and ITS B. The ITS/5.8S sequences produced include all but the first ca. 10 bp of ITS 1 and the entire ITS 2 and 5.8S regions. Rarely, only ITS 1 or ITS 2 was obtained.

PCR products were purified following the protocol provided by QIAquick gel extraction kits (QIAGEN) and sequenced using the dye terminator cycle sequencing protocol (Applied Biosystems). Sequence reactions were analyzed on ABI 373 or ABI 377 automated sequencers (University of Missouri-Columbia DNA Core Sequencing Facility and University of Missouri-St. Louis D. E. Lee and Family Sequencing Facility). Except for the *psb*A-*trn*H region, which could only be sequenced from the 5′ end, both strands of DNA were read and

consensus sequences generated using Sequencher vers. 3.1 (Gene Codes Corp., 1998). Sequences were manually aligned using the sequence editing facilities of Seqpup VERSION 0.6 (D. Gilbert, Indiana University, Bloomington, 1996). Sequence alignment was relatively straightforward in Lauraceae. However, the first 290 bp of the *trn*T-*trn*L spacer were removed from analysis because of alignment difficulties among basal Lauraceae and outgroup families. Alignment difficulties with the outgroup were also encountered in the 3′ half of the *psb*A-*trn*H spacer. However, unlike *trn*T-*trn*L sequences, *psb*A-*trn*H provided several informative characters within Lauraceae and instead of removing it entirely, outgroup sequences were truncated after alignment became ambiguous (ca. position 300).

PHYLOGENETIC ANALYSES

Initial analyses of the individual data sets were conducted as heuristic searches for most parsimonious trees with 10 random taxon additions and TBR branch swapping using PAUP* version 4.0b4 (Swofford, 1998). Both the MULPARS and COLLAPSE options were in effect, but the STEEPEST DESCENT option was not employed. Characters were assumed to be unordered (i.e., Fitch parsimony), equally weighted, and gaps were treated as missing data. Parsimony uninformative characters were excluded. Bootstrap analyses (Felsenstein, 1985) with 500 replications were performed with the above heuristic search settings but with MAXTREES set to 100. Since these initial searches showed no strongly supported conflict, i.e., alternative clades supported by > 70% bootstrap values (Hillis & Bull, 1993), and P-values from partition-homogeneity tests ranged from 1.0 to 0.6 (strongly indicating congruence), three matrices were compiled for further analyses. Matrix I combined 86 species that were sequenced for the *trn*L-*trn*F and *psb*A-*trn*H regions. Matrix II combined *trn*L-*trn*F, *psb*A-*trn*H, *trn*T-*trn*L, *rpl*16, and 26S sequences for 42 taxa representing major lineages identified by analyses of matrix I to further investigate basal relationships. Matrix III included 94 ITS sequences from representatives of a large terminal clade that was poorly resolved by sequence variation in matrix I. The only mutation in matrix I that provided generic-level information in this terminal clade, a 16-bp repeat in *trn*L-*trn*F, was included in matrix III. Analyses of all three matrices were conducted as above with length mutations (insertions and deletions) introduced as binary characters of equal weight. In addition, minimum evolution topologies were calculated for the ITS data set using log determinant (LogDet) and maximum likelihood estimates of genetic distances. Maximum likelihood distances were calculated using the Hasegawa-Kishino-Yano (1985) model with rate heterogeneity among sites (HKY−Γ).

MOLECULAR CLOCK ANALYSES

In order to add a temporal dimension to the phylogenetic estimate, divergence times for major branches were estimated. Due to the computational demands of maximum likelihood calculations and because it was not necessary to obtain divergence times for all nodes, likelihood scores were obtained from parsimony-based topologies that were reduced to exemplars of major clades. ITS/5.8S sequences were used for terminal, and the cpDNA and 26S sequences for basal lineages.

Likelihood scores were calculated under the Hasegawa-Kishino-Yano (1985) model with rate heterogeneity among sites, and values obtained with and without a clock constraint compared for significance using a χ^2 test [$\chi^2 = 2(\log L_{clock} - \log L_{nonclock})$; d.f. = number of taxa − 2]. For each internal node, HKY−Γ distances (branch lengths) to the tip were computed by PAUP* with the clock constraint enforced. These values represent the number of substitutions per site accumulated along each daughter lineage, and were either divided by time to determine substitution rates, or divided by estimated rates to obtain divergence times. Overlap in taxon sample between terminal and basal topologies allowed use of divergence times estimated in one to calibrate substitution rates and calculate divergence times in the other. Standard deviations of divergence times were estimated as follows. First, standard deviations of HKY-Γ distances were calculated using a formula derived from the relationship S = Np, where S is the number of nucleotide substitutions, N is the total number of nucleotide positions in a sequence, and p is the proportion of nucleotides substituted (HKY-Γ distance from node to branch tips). Since the standard deviation of S is the square root of Np(1 − p), or SD(S) = sqrt(Np[1 − p]), the standard deviation of p is SD(S) divided by N, or SD(p) = sqrt(p[1 − p]/N). This value is calculated for each divergence of interest and divided by substitution rate to obtain the standard deviation of divergence times.

RESULTS

PHYLOGENETIC ANALYSES

Sequence variation in chloroplast markers and partial 26S sequences was almost limited to basal

Table 2. Characteristics and comparisons of phylogenetic information provided by matrices analyzed in this study. Matrix I combines *trn*L-*trn*F and *psb*A-*trn*H cpDNA sequences, Matrix II combines *trn*L-*trn*F, *trn*T-*trn*L, *psb*A-*trn*H, and *rpl*16 of cpDNA with 26S rDNA sequences; Matrix III is based on ITS rDNA sequences.

		Number of representatives of:		Parsimony-informative substitutions among:	
Matrices	Aligned length	Lauraceae	Perseeae & Laureae	Lauraceae	Perseeae & Laureae
*trn*L-*trn*F	510	76	48	103	7
*psb*A-*trn*H	616	75	48	135	35
*trn*T-*trn*L	530	33	19	105	12
*rpl*16	1049	24	10	103	9
26S	592	22	8	77	11
Matrix I	1126	77	48	238	42
Matrix II	3297	42	4	470	n/a
Matrix III	780	94	90	n/a	199

branches in Lauraceae with very little and often no variation among members of tribes Perseeae and Laureae (sensu van der Werff & Richter, 1996). The *trn*L-*trn*F matrix included 510 aligned positions and yielded 103 informative substitutions within Lauraceae, but only seven of these were informative among Perseeae and Laureae. Similarly, of 616 aligned positions in the *psb*A-*trn*H matrix, 135 substitutions were parsimony informative within Lauraceae and 35 among Perseeae and Laureae. This trend was also found in other chloroplast loci and partial 26S sequences. Only ITS showed substantial variation within Perseeae and Laureae. Characteristics of individual genetic markers and combined data sets are summarized in Table 2.

Combined *trn*L-*trn*F and *psb*A-*trn*H matrix (matrix I) included 1126 aligned positions of which 277 were informative. Four indels from *trn*L-*trn*F were added as binary characters. Three of these supported a clade comprised of *Beilschmiedia*, *Cryptocarya*, *Endiandra*, and *Potameia*, and the fourth is a 16-base pair repeat found in several neotropical genera of Perseeae and *Umbellularia* of Laureae. Parsimony analyses of matrix I surpassed memory limitations in the first addition replicate, and 29,000 equally parsimonious topologies were retained after 24 hours of branch swapping (L = 796, CI = 0.74, RI = 0.89). The strict consensus topology (Fig. 1) shows two well-supported clades in Lauraceae. One of these includes members of Cryptocaryeae (sensu van der Werff & Richter, 1996), with *Hypodaphnis* tenuously placed as its sister group. The second major clade is unequally divided into a small clade of South American genera (hereafter *Chlorocardium–Mezilaurus* clade) and a large terminal clade comprised of representatives of Laureae and Perseeae (sensu van der Werff & Richter, 1996). This Perseeae–Laureae clade is essentially unresolved but there is some support for a clade including representatives of *Persea* and *Alseodaphne*, a large generic complex centered around *Ocotea*, and strong support for a clade including all Asian members of *Cinnamomum*. Furthermore, a clade uniting most neotropical genera (*Aniba*, *Endlicheria*, *Kubitzkia*, *Licaria*, *Nectandra*, *Pleurothyrium*, and *Rhodostemonodaphne*) with neotropical (but not paleotropical) species of *Ocotea* and Californian *Umbellularia* is recovered in all trees but does not receive strong support. All members of this clade, except *O. leucoxylon* (Sw.) Laness., have the 16-bp repeat mentioned above. *Caryodaphnopsis*, *Cassytha*, and *Neocinnamomum* constitute a clade in all trees but this association does not receive more than 50% bootstrap support, and neither does its position as the sister group of the second major infrafamilial group.

Matrix II included 3304 characters, of which 684 were parsimony-informative substitutions and 7 were binary-coded length mutations. Parsimony analysis converged on a single island of 2646 equal length trees (L = 2171, CI = 0.71, RI = 0.72), the majority rule consensus of which is shown in Figure 2. The topology is mostly congruent with that provided by matrix I, differing mainly in that *Hypodaphnis* occupies a fairly well supported position (found in 81% of the bootstrap replicates) sister to the rest of the family. The placement of *Caryodaphnopsis*, *Cassytha*, and *Neocinnamomum* in the second main clade receives very strong (98%) bootstrap support, and relationships within the *Chlorocardium–Mezilaurus* clade are altered slightly.

Matrix III, based mainly on ITS sequences, included 259 parsimony-informative substitutions and 19 length mutations that could be unambiguously coded. Parsimony analysis found a single is-

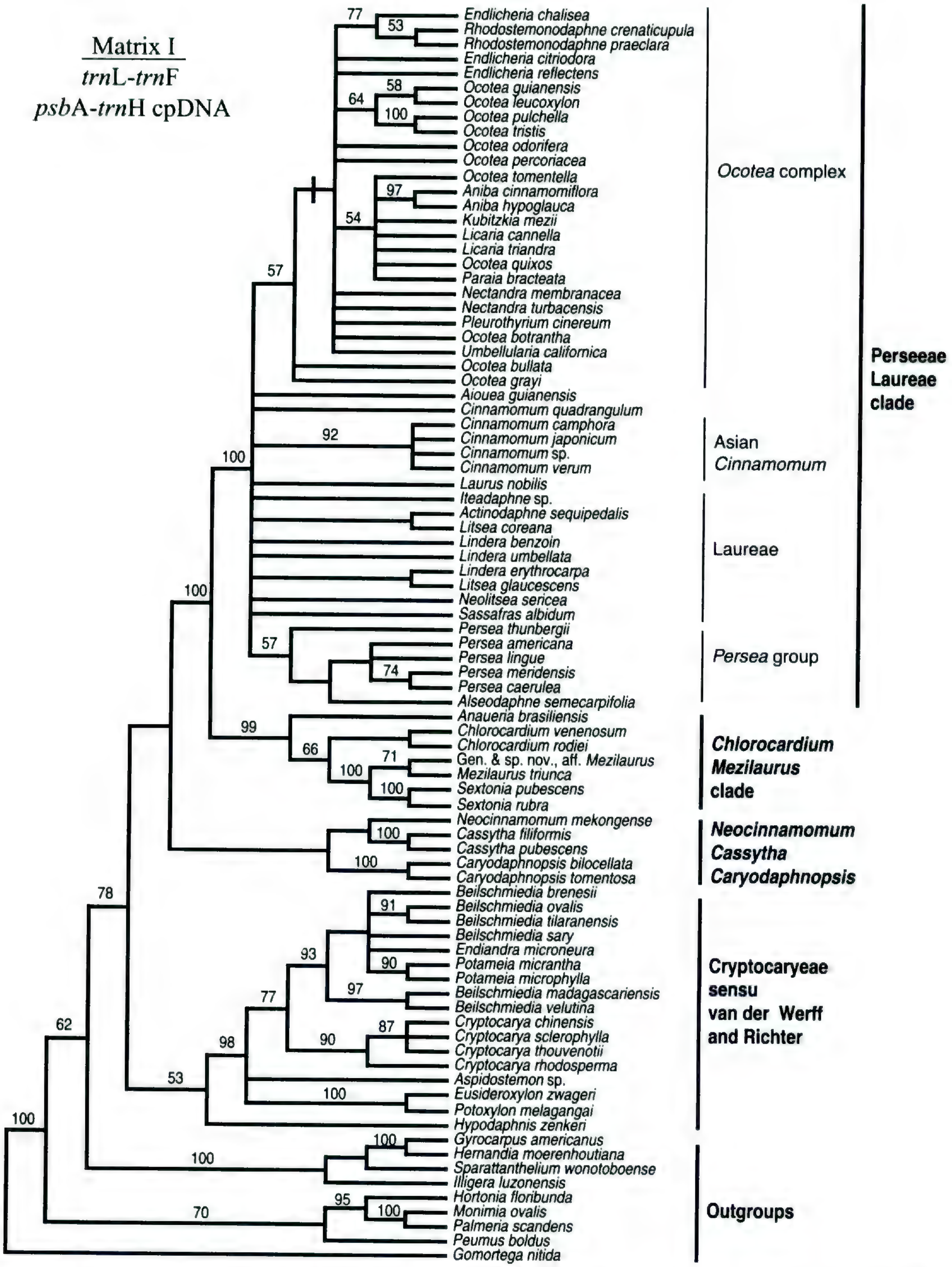


Figure 1. Strict consensus of 29,000 trees retained from parsimony analysis of matrix I (*trn*L-*trn*F and *psb*A-*trn*H cpDNA sequences). Numbers above branches indicate bootstrap support. The vertical bar through the branch supporting the terminal clade indicates a 16-bp repeat in the *trn*L-*trn*F intergenic spacer that unites taxa therein.

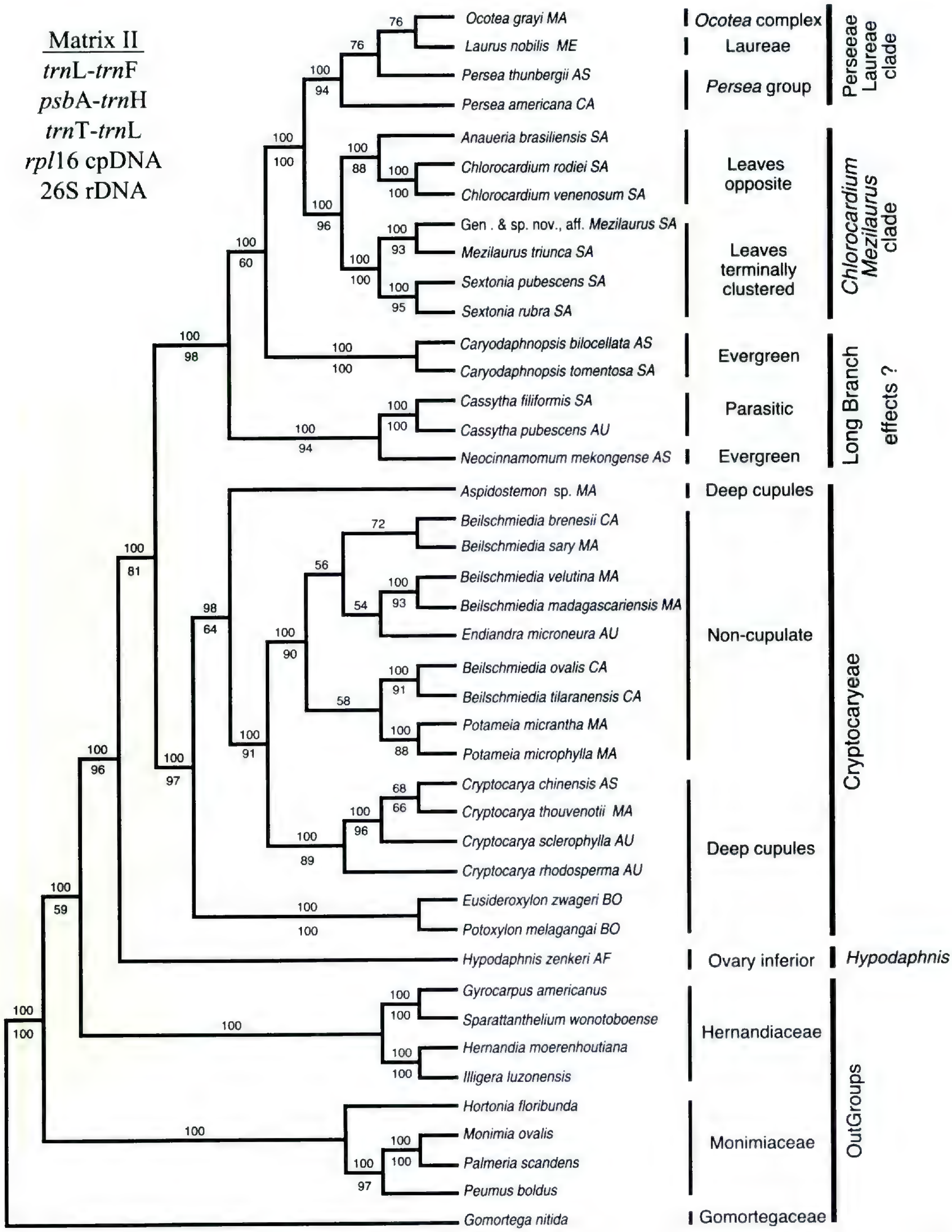


Figure 2. Annotated 50% majority rule tree resulting from parsimony analysis of matrix II (*trn*L-*trn*F, *psb*A-*trn*H, *trn*T-*trn*L, *rpl*16, and 26S sequences). Majority rule percentages are indicated above, and bootstrap values > 50% below, branches. Main clades and significant morphological characters are indicated. Geographic regions represented by each species of Lauraceae are given: AF = Africa, AS = Asia, AU = Australia, BO = Borneo, CA = Central America, MA = Madagascar, ME = Mediterranean, and SA = South America.

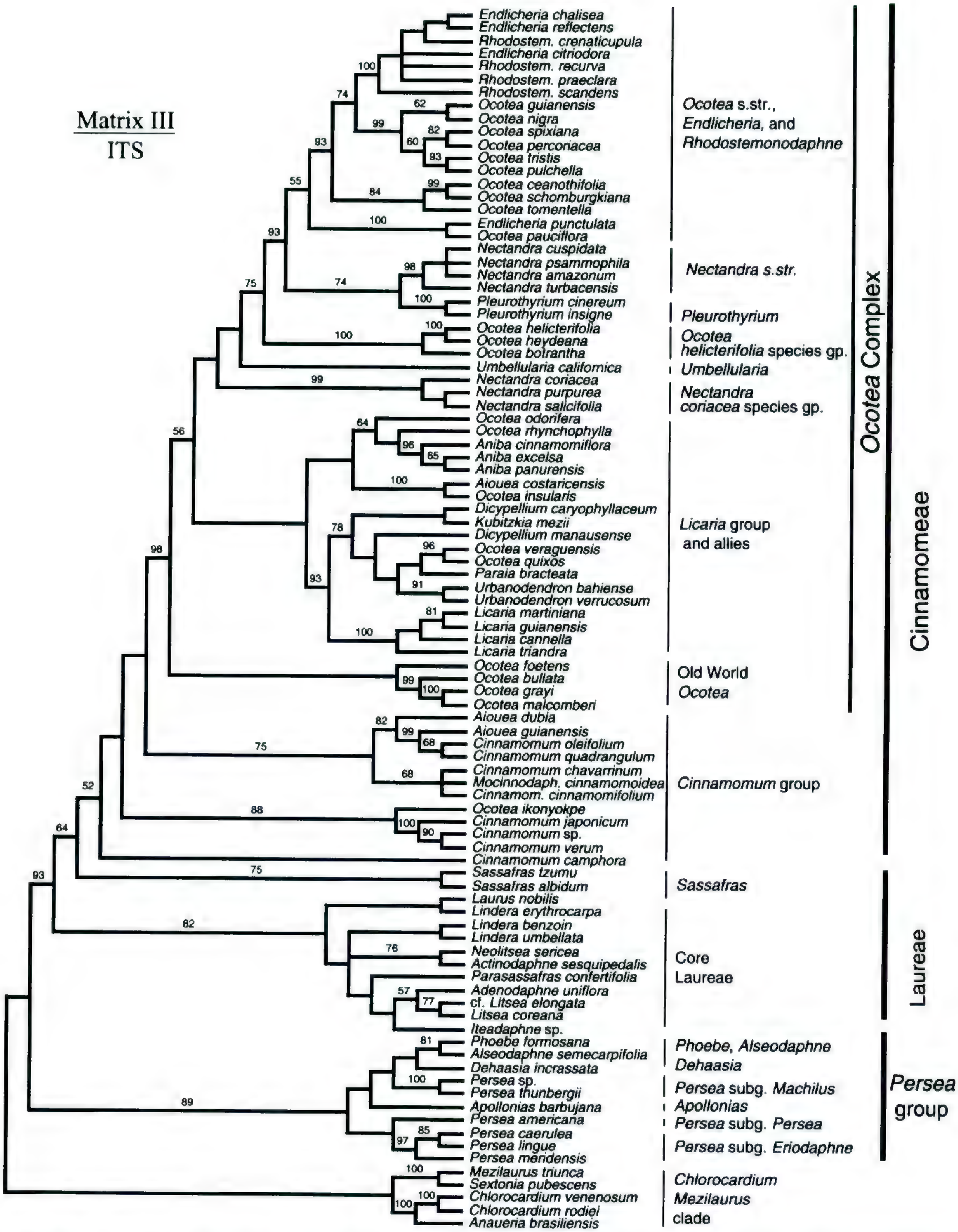


Figure 3. Adams consensus of 567 equally parsimonious trees obtained from unconstrained analysis of matrix III (ITS sequences). Numbers above branches indicate bootstrap support, and vertical bars to the right circumscribe main clades. *Cinnamom.* = *Cinnamomum*, *Mocinnodaph.* = *Mocinnodaphne*, and *Rhodostem.* = *Rhodostemonodaphne*.

land of 567 equal length trees (L = 1050, CI = 0.44, RI = 0.75). The Adams consensus topology (Fig. 3) indicates that Perseeae sensu van der Werff and Richter (1996) are paraphyletic, and that most of the topological instability exists among members of *Cinnamomum*. *Sassafras* was placed between Laureae and a paraphyletic *Cinnamomum* in all trees. This reconstruction does not receive strong

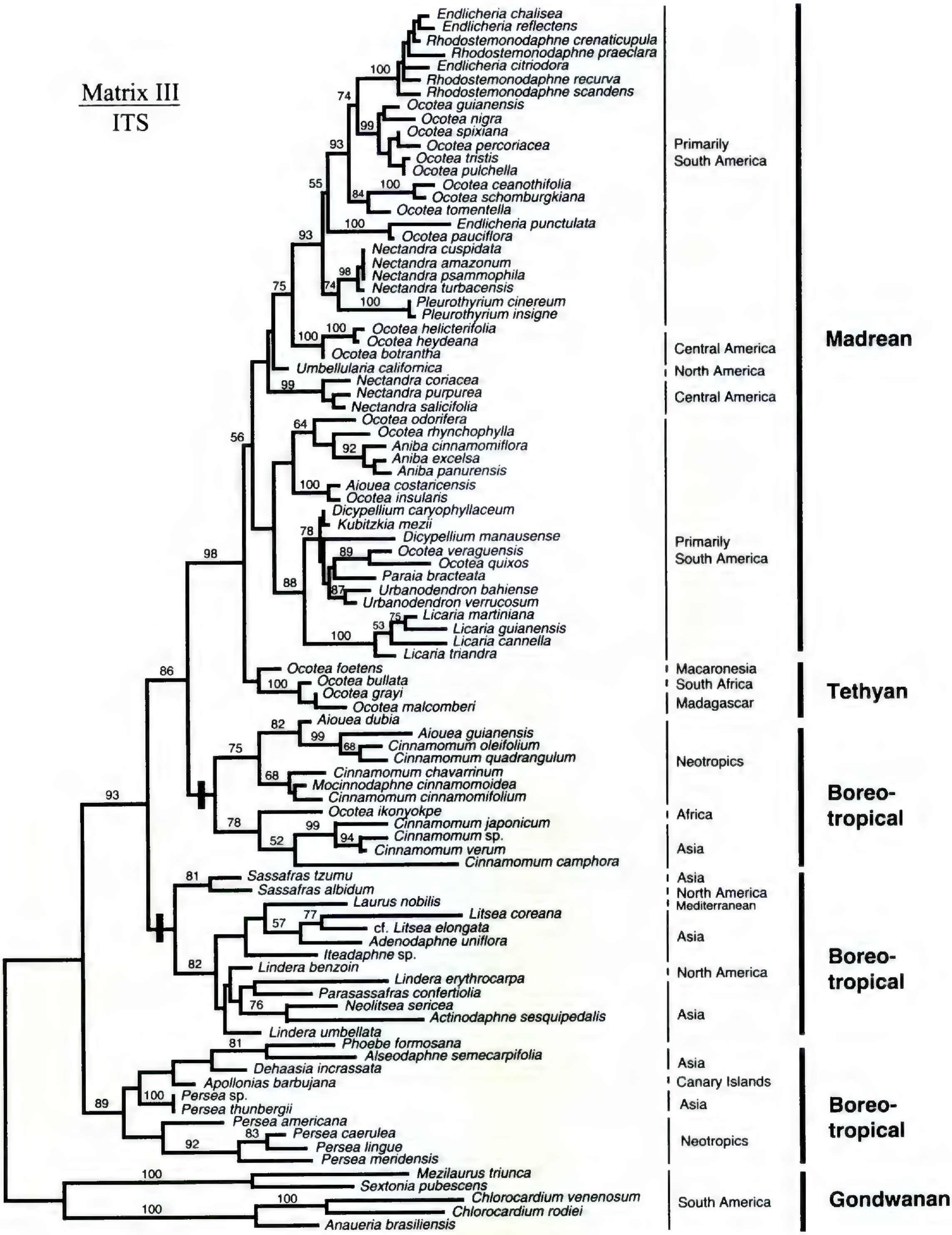


Figure 4. One of 126 equally parsimonious trees obtained with monophyly of the *Cinnamomum* group (*Cinnamomum*, *Aiouea* p.p., *Mocinnodaphne*, and *Ocotea* p.p.) and Laureae enforced (thick vertical bars) as topological constraints. Bootstrap support values are indicated above unconstrained branches. Annotated bars to the right indicate the distribution and inferred ancestry of clades.

bootstrap support, and constraining monophyly of a clade comprised of *Cinnamomum* and its allies (*Aiouea* p.p., *Mocinnodaphne, Ocotea* p.p.) required only one extra step while constraining monophyly of Laureae to include *Sassafras* added three extra steps to parsimony-based trees. One of 126 equally parsimonious trees (L = 1054, CI = 0.44, RI = 0.75) calculated with both topological constraints enforced is shown in Figure 4. Both minimum-evolution analyses recovered topologies that showed the same main clades found by parsimony analyses. Although *Cinnamomum* was again not monophyletic, minimum evolution placed *Sassafras* sister to remaining Laureae, albeit together with *Cinnamomum camphora* (L.) Presl. Differences between parsimony and minimum evolution reconstructions suggest that some instability in parsimony-based topologies can be attributed to characters shared between *Sassafras* and *C. camphora.* With *C. camphora* removed the number of trees resulting from unconstrained parsimony analyses is greatly reduced (to 36, L = 1022 CI = 0.44, RI = 0.76), but *Sassafras* is still placed between Laureae and paraphyletic *Cinnamomum.*

MOLECULAR CLOCK ANALYSES

A likelihood ratio test on the ITS data set reduced to 25 representatives of main lineages in higher Lauraceae (Fig. 5a) indicated that substitution was approximately clock-like. Log likelihood scores with (−2844.00) and without a clock constraint (−2827.85) were not significantly different ($\chi^2 = 32.30$, d.f. = 23, $P > 0.05$). Of molecular markers used to resolve basal relationships, only *rpl*16 did not reject the molecular clock [$\chi^2 = 2(2590.76 - 2582.73) = 16.06$; d.f. = 10, $P > 0.05$], provided *Neocinnamomum* and *Cassytha* were removed from analysis (Fig. 5b). Results of two calibrations simulating alternative biogeographic scenarios are summarized in Table 3, and those of our preferred calibration are depicted in Figure 5.

DISCUSSION

The two-tiered taxon and molecular sampling adopted in this study provides a generally well supported generic-level phylogeny for Lauraceae. Data from chloroplast markers and partial 26S sequences resolve main clades, while ITS provides novel resolution among members of Perseeae and Laureae (sensu van der Werff & Richter, 1996). To provide a basis for subsequent biogeographic considerations, phylogenetic relationships among Lauraceae are first discussed.

PHYLOGENETIC RELATIONSHIPS

Several previously recognized taxonomic groups, albeit in different schemes, compare favorably with clades supported by our molecular data. Among these, Cryptocaryeae as circumscribed by van der Werff and Richter (1996), Laureae of most classifications (e.g., Kostermans, 1957; van der Werff & Richter, 1996; Rohwer, 1993a), and Cinnamomeae in the sense of Kostermans (1957) are the largest. In addition, a generic grouping centered around *Persea,* informally recognized by Rohwer (1993a), herein receives considerable support. A fifth major generic grouping, the *Chlorocardium–Mezilaurus* clade, is comprised of taxa whose taxonomic positions have previously been uncertain. Outside of these main clades the position of a few small genera is unsettled.

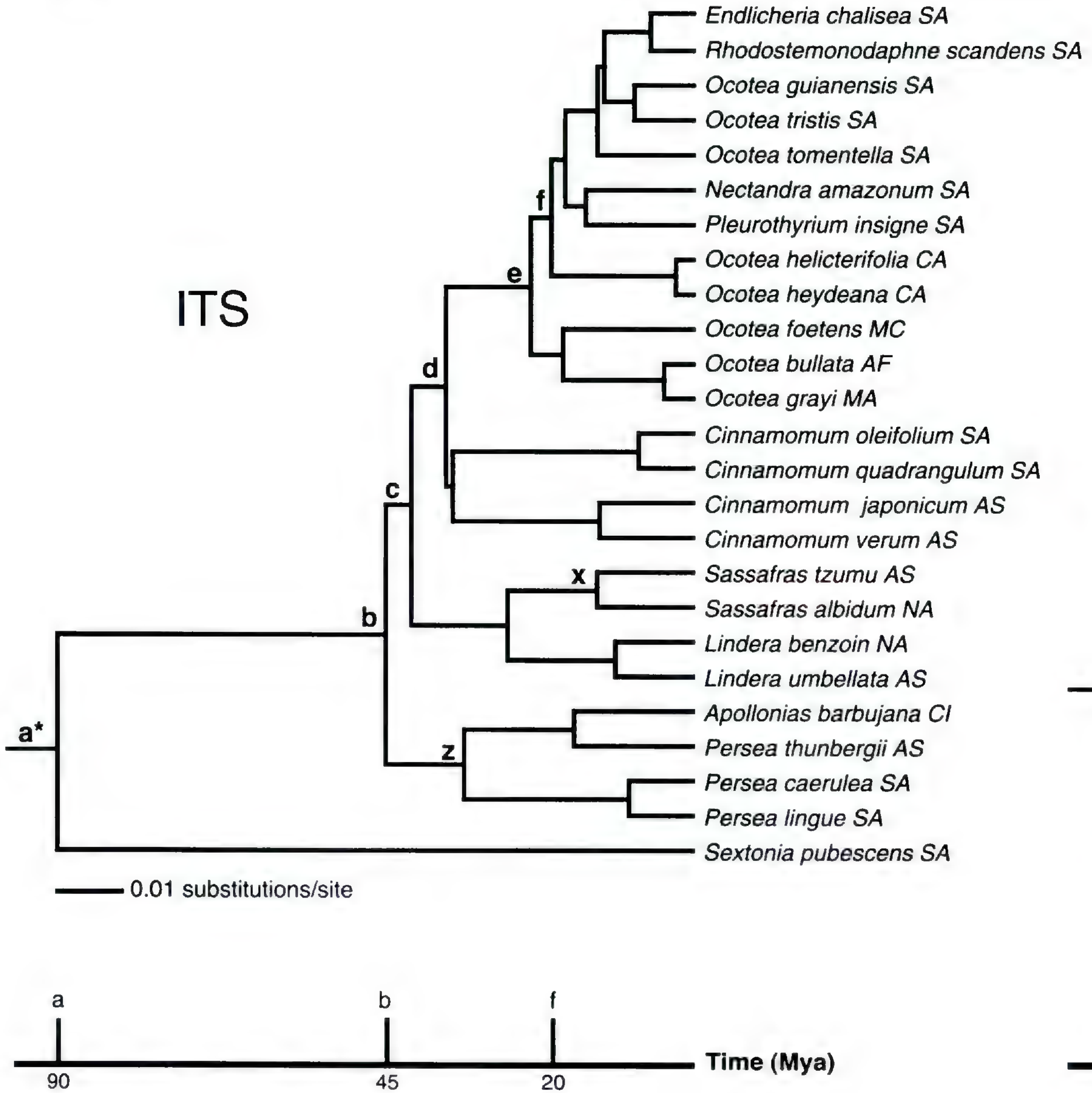
Hypodaphnis *and* Cryptocaryeae. Monotypic *Hypodaphnis,* consisting of *H. zenkeri* (Engl.) Stapf from Central Africa, is the only member of Lauraceae with an inferior ovary, and the two analyses that investigated basal relationships in Lauraceae suggested different positions (compare Figs. 1 and 2). The *trn*L-*trn*F and *psb*A-*trn*H data sets analyzed in matrix I weakly support a sister group relationship between *Hypodaphnis* and Cryptocaryeae (Fig. 1). Association with Cryptocaryeae is supported by irregular thyrsoid inflorescences (van der Werff & Richter, 1996) and morphological similarity with *Eusideroxylon* and *Potoxylon,* two monotypic Indonesian genera that consistently place basally in Cryptocaryeae (Figs. 1, 2). Like *Hypodaphnis,* they have stamens with four collaterally arranged locelli, but their ovaries are only semi-inferior. However, the larger molecular sample (matrix II) places *Hypodaphnis* sister to remaining Lauraceae with mod-

→

Figure 5. Phylograms showing clock enforced HKY-Γ distances on reduced parsimony-based topologies depicting terminal (5a) and basal (5b) clades in Lauraceae. Divergence times in Figure 5a are based on ITS and those in Figure 5b on *rpl*16 sequences. The time scale below each phylogram reflects a calibration in which equivalent nodes (indicated by *) in Figure 5a and Figure 5b are fixed at 90 million years. Node labels (a–f, x, z, and A–G) correspond to those listed in Table 3. The geographic distributions of terminal taxa are given: AF = Africa, AS = Asia, BO = Borneo, CA = Central America, CI = Canary Islands, MA = Madagascar, MC = Macaronesia, NA = North America, and SA = South America.

5a

ITS



5b

*rpl*16

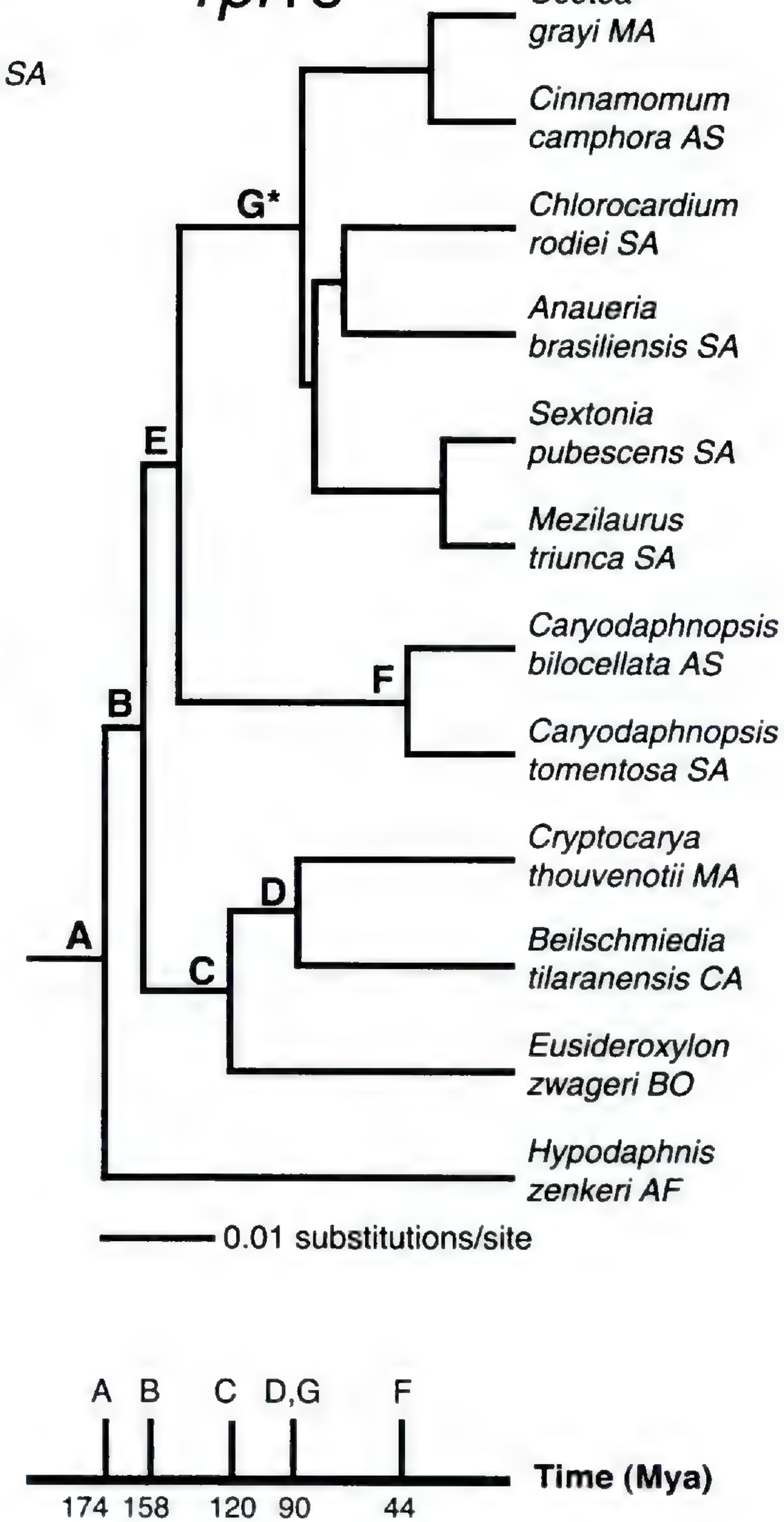


Table 3. Clock enforced HKY-Γ, distances accumulated after divergence events among terminal (Table 3a) and basal (Table 3b) lineages in Lauraceae, and estimates of divergence times simulating two biogeographic scenarios. HKY-Γ, distances in Table 3a are obtained from ITS and those in Table 3b from *rpl*16 sequences. Calibration 1 simulates West Gondwanan vicariance for the trans-Atlantic disjunction in the *Ocotea* complex; calibration 2 simulates Gondwanan origin for the *Chlorocardium–Mezilaurus* clade. Node labels are equivalent to those in Figure 5, and ages fixed for calibration purposes are underlined. * = Equivalent nodes.

Node	HKY-Γ distance from node to tip ± SD	Calibration 1 time (Mya) ± SD	Calibration 2 time (Mya) ± SD
a. Terminal lineages in Lauraceae.			
a*	0.09740 ± 0.0106	354 ± 30	90
b	0.05715 ± 0.0083	171 ± 21	44 ± 7
c	0.04309 ± 0.0073	156 ± 20	40 ± 7
d	0.03793 ± 0.0068	138 ± 19	35 ± 6
e	0.02476 ± 0.0055	90	23 ± 5
f	0.02175 ± 0.0052	79 ± 14	20 ± 5
x	0.01437 ± 0.0042	52 ± 12	13 ± 4
z	0.03499 ± 0.0066	127 ± 18	32 ± 6
b. Basal lineages in Lauraceae.			
A	0.03886 ± 0.0059	682 ± 105	174 ± 32
B	0.03513 ± 0.0056	620 ± 100	158 ± 31
C	0.02683 ± 0.0050	473 ± 88	120 ± 27
D	0.02038 ± 0.0044	360 ± 77	91 ± 20
E	0.03162 ± 0.0054	558 ± 95	142 ± 24
F	0.00977 ± 0.0058	172 ± 54	44 ± 14
G*	0.02006 ± 0.0043	354	90

erately high support (Fig. 2). This peripheral position for *Hypodaphnis* is also indicated by *mat*K sequences, albeit with < 50% bootstrap support (Rohwer, 2000), but difficult to support with morphology. Any outgroup comparison is stymied by the unsettled sister family relationship of Lauraceae. Hernandiaceae, with inferior ovaries, are favored over Monimiaceae with 100% bootstrap support by morphology (Doyle & Endress, 2000), but molecular data have been ambiguous (Renner, 1999; Renner & Chanderbali, 2000; Qiu et al., 1999). A Hernandiaceae–Lauraceae clade receives modest support here (Figs. 1, 2), and when *Hypodaphnis* lies sister to remaining Lauraceae (Fig. 2), inferior ovaries are a potential synapomorphy for the two families. However, given the non-inferior state of all other Lauraceae, independent gain, required also by the topology in Figure 1, is equally parsimonious.

Cryptocaryeae sensu stricto. Support for the clade comprised of *Aspidostemon, Beilschmiedia, Cryptocarya, Endiandra, Eusideroxylon, Potameia,* and *Potoxylon* is considerable in both analyses investigating basal relationships in Lauraceae (Figs. 1, 2). Anatomical features stress isolation of *Aspidostemon, Eusideroxylon,* and *Potoxylon* more than their affinities (Richter, 1981), but close relationship with *Cryptocarya* has been suggested in recent morphology-based systems (e.g., Rohwer, 1993a; van der Werff & Richter, 1996). With *Cryptocarya* they share a deeply urceolate floral hypanthium that develops into deep cupules enclosing the drupe except for a small terminal orifice. Unlike the previous genera, in *Beilschmiedia* and *Endiandra* the hypanthium is shallow and a cupule never develops, while the fruits of *Potameia* are either free or seated in a small discoid structure. Kostermans (1957), stressing the degree of cupule development in his scheme for Lauraceae, placed *Beilschmiedia, Endiandra,* and *Potameia* with *Persea* (also non-cupulate, but of the distal Perseeae–Laureae clade). Close relationship with *Cryptocarya* has since gained support from wood and bark anatomy (Richter, 1981), inflorescence morphology (van der Werff & Richter, 1996), embryology (Heo et al., 1998), and molecular data (Rohwer, 2000; herein).

The topology in Cryptocaryeae reveals a trend toward increased ovary exsertion, in both flower and fruit. *Eusideroxylon* and *Potoxylon,* with semi-inferior ovaries, lie sister to genera with superior ovaries. *Aspidostemon* branches next (Fig. 2), and *Cryptocarya* lies sister to the non-cupulate clade of *Beilschmiedia, Endiandra,* and *Potameia* (Figs. 1, 2). *Endiandra* and *Potameia* have a reduced number of floral parts relative to *Beilschmiedia,* but whether they nest within the latter (Figs. 1, 2) is not well resolved.

Other genera that have been allied to members of Cryptocaryeae but not herein are either monotypic or oligotypic. Their generic status is also controversial (e.g., Rohwer, 1993a). *Dahlgrenodendron,* with only *D. natalensis* (J. H. Ross) J. J. M. van der Merwe & A. E. van Wyk, has distinctive pollen ornamentation (van der Merwe et al., 1988) but is otherwise not different from *Cryptocarya* (Rohwer, 1993a). *Triadodaphne,* with three species, is tentatively distinguished from *Endiandra* by its unequal perianth whorls and deeper hypanthium (Kostermans, 1993). In *Hexapora,* comprised of *H. curtisii* Hook. f., the outer six stamens are extrorse, and the third and fourth whorls staminodial, but otherwise the genus is similar to *Beilschmiedia. Brassiodendron,* with only *B. fragrans* C. K. Allen, also has only six fertile stamens, and according to Kostermans (1957) and Hyland (1989) should be included in *Endiandra.*

Cassytha, Caryodaphnopsis, *and* Neocinnamomum. These three genera are among the most enigmatic of the family. In our analyses of matrix I, they constitute a statistically uncorroborated clade (with < 50% bootstrap support) that lies sister to the rest of the family, also without support (Fig. 1). This alliance is disbanded by additional molecular characters provided by matrix II, but their position in this part of the tree receives strong support (Fig. 2). Some elements of *Cassytha*'s position may be due to long branch effects in our analyses.

The herbaceous parasitic twiner, *Cassytha*, is the sole exception to the arborescence typical of Lauraceae, and it has usually been placed in a separate subfamily (e.g., Kostermans, 1957; van der Werff & Richter, 1996). Subfamilial position is supported by ab initio cellular endosperm *Cassytha* shares with Hernandiaceae, Monimiaceae, and other Laurales (Heo & Tobe, 1995; Heo et al., 1998). Such endosperm formation is also reported from *Umbellularia* (Bambacioni-Mezzetti, 1941), but nuclear endosperm is found in all other Lauraceae that have been examined, including *Hypodaphnis* (Heo et al., 1998). In Rohwer's *mat*K study *Cassytha* was placed between *Hypodaphnis* and the rest of the family, but without strong statistical support. Here, close relationship with *Neocinnamomum* receives very high statistical support from matrix II (Fig. 2), but morphological synapomorphies for these two genera are not known. Instead, *Cassytha* and *Neocinnamomum* have the longest branches in the topology, differing from each other by over 279 mutations (uncorrected "p" distances, uninformative characters included), while the branch uniting them is supported by comparatively few (66) mutations. With *Neocinnamomum* removed from the analysis, *Cassytha* and *Caryodaphnopsis* constitute a clade (cladogram not shown). In both genera the outer whorl of tepals is strongly reduced, but this condition appears elsewhere in Lauraceae (e.g., *Persea*), and other characters to support a *Cassytha*–*Caryodaphnopsis* clade are unknown. However, with both *Neocinnamomum* and *Caryodaphnopsis* removed, *Cassytha* still lies sister to the rest of the family. If long branches cannot attract in their mutual absence (Sidall & Whiting, 1999), this finding would imply that *Cassytha* is correctly placed in this general part of the phylogeny. Alternatively, long branched taxa, such as *Cassytha*, experience multiple substitutions that erode genealogical signal, randomize character states with respect to true relatives, and lead to chance convergence on the molecular states of distant lineages, all qualities that can mislead phylogenetic estimates (Felsenstein, 1978; Lyons-Weiler & Hoelzer, 1997).

Caryodaphnopsis and *Neocinnamomum* are morphologically similar, sharing triplinerved venation and four-locular anthers with the locelli arranged in a shallow arc (sometimes two-locular in *Caryodaphnopsis*, in a horizontal row in *Neocinnamomum delavayi* (Lecomte) H. Liu). In contrast, *Caryodaphnopsis* has opposite leaves, a perianth of strongly unequal tepals, and lacks a cupule, while *Neocinnamomum* has alternate (spiral) leaves, subequal tepals, and a shallow cupule with persistent tepals. Close relationship between *Neocinnamomum* and *Cinnamomum* (Kostermans, 1974a), and between *Caryodaphnopsis* and *Persea* (Kostermans, 1974b; Rohwer, 1993a), can be ruled out, but the relationships of these two genera are not clearly indicated by our data. They either constitute a clade, albeit with *Cassytha* (Fig. 1), or *Caryodaphnopsis* lies between a *Neocinnamomum–Cassytha* clade and the rest of the family (Fig. 2). With *Cassytha* excluded, either *Caryodaphnopsis* or *Neocinnamomum* lies sister to the rest of the family (cladograms not shown). *Neocinnamomum* was not included in Rohwer's *mat*K study, and *Caryodaphnopsis* was placed as it is in Figure 2. Anatomical affinities of *Neocinnamomum* and *Caryodaphnopsis* with *Chlorocardium* and Cryptocaryeae, respectively (Richter, 1981), are consistent with their relatively basal position in the family.

Chlorocardium–Mezilaurus *clade*. The clade comprised of *Anaueria*, *Chlorocardium*, *Mezilaurus*, *Sextonia*, and a novel taxon (Gen. & sp. nov.) receives 99% and 96% bootstrap support from matrix I and II, respectively (Fig. 1). It is one of the more intriguing clades in the family. Close relationship between *Anaueria* and *Mezilaurus* has been suggested (Richter, 1981; Rohwer, 1993a), but the possibility that all these taxa constitute a clade of their own has never been considered on morphological grounds. So far, characters uniting them have only been provided by molecular data. A clade comprised of *Chlorocardium* and *Mezilaurus* (plus *Williamodendron*, a small genus of 3 species differing from *Mezilaurus* primarily in the number of locelli; not herein) received moderate support from *mat*K sequences, but Rohwer (2000) questioned a close relationship citing anatomical and floral differences. Here, *Chlorocardium* and *Mezilaurus* place in separate subclades that can be characterized by phyllotaxy.

The subclade of Gen. & sp. nov., *Mezilaurus*, and *Sextonia* accommodates taxa with obovate, coriaceous leaves borne in terminal clusters (Fig. 2).

Unlike most other Lauraceae with terminally clustered leaves, in these taxa the clusters are not separated by seasonal growth spurts. Instead, growth is continuous, and apparently quite slow, resulting in a continuous spiral of leaf scars. This growth pattern is rare but not unique to this clade; such leaf clusters are at least also known in *Alseodaphne*, of the *Persea* group (below). In the other subclade, *Anaueria* and *Chlorocardium* share opposite leaves (Fig. 2). This subclade receives considerable molecular support, but both genera find morphological allies within the other subclade. *Anaueria* is anatomically more similar to *Mezilaurus* (Richter, 1981), with which it also shares glandless flowers. *Chlorocardium* is unique with its xylem of coronated vessel elements (Kostermans et al., 1969), and its large rotate flowers with an increased number of stamens (to 20) are unmatched in the clade. However, its papillose, tongue-shaped stamens, all with a pair of small basal glands, are much like those found in the second and third staminal whorls of *Sextonia*.

All South American genera that do not clearly assign to generic groups based on wood and bark anatomy or inflorescence structure (van der Werff & Richter, 1996) place in a *Chlorocardium–Mezilaurus* clade. Monotypic Costa Rican *Povedadaphne* (not examined here) was considered close to *Mezilaurus* by Rohwer (1993a), but his *mat*K data (Rohwer, 2000) suggested a place with genera here placed in Cinnamomeae (below) where it is morphologically close to the *Ocotea* complex. No other members of the *Chlorocardium–Mezilaurus* clade are suspected on morphological grounds.

Monophyletic Groups in the Perseeae–Laureae clade. The large terminal clade that accounts for most of the modern generic and species diversity of Lauraceae includes Laureae of most classification systems and van der Werff and Richter's (1996) Perseeae. The present resolution does not support the dichotomy implied by these tribal concepts (Fig. 3). Instead, five genera of Perseeae (*Persea* group) lie sister to a clade comprised of Laureae and remaining Perseeae. The generic composition of the latter group compares favorably with Kostermans' (1957) Cinnamomeae (Fig. 3). Possible resolution of a Perseeae–Laureae clade into a small *Persea* group and a large Laureae–Cinnamomeae clade was indicated by *mat*K sequences (Rohwer, 2000), but topology was unstable and lacked statistical support. Support is stronger here with the *Persea* group and Laureae–Cinnamomeae clade receiving 89% and 93% bootstrap support, respectively (Figs. 3, 4). A close Laureae–Cinnamomeae relationship has never been formally recognized. Only Kostermans (1957) placed these two tribes in close proximity in his graphical scheme for Lauraceae, conceivably to express his observation that fleshy hemispherical cupules are typical of, and restricted to, genera therein. Elsewhere in Lauraceae hemispherical, but rather woody, cupules also occur in *Chlorocardium* and *Sextonia*, both included in Cinnamomeae by Kostermans (as *Ocotea*), but of the *Chlorocardium–Mezilaurus* clade herein.

Persea *group.* This clade (Fig. 3), including *Alseodaphne*, *Apollonias*, *Dehaasia*, *Persea*, and *Phoebe*, accommodates all non-cupulate genera of van der Werff and Richter's (1996) Perseeae. It is Rohwer's (1993a) *Persea* group, without *Caryodaphnopsis*. *Nothaphoebe* was not investigated herein but is morphologically close to *Alseodaphne*.

Generic delimitation in the *Persea* group has been controversial, and all genera with four-locular anthers have at some point been placed in synonymy under *Persea* (e.g., Bentham, 1880; Kostermans, 1957). *Apollonias* and *Dehaasia* have always been segregated on the basis of their two-locular anthers, but the generic importance of this character and its use to delimit *Apollonias* from *Phoebe* and *Dehaasia* from *Alseodaphne* is questionable (Rohwer et al., 1991; Rohwer, 1993a; van der Werff, in press). Other generic characters, including relative tepal sizes, whether tepals persist in fruit, and if so, manner of persistence, have also been questioned (van der Werff, 1989; Rohwer et al., 1991). Our present sampling does not adequately address these issues. Only *Persea* is represented by more than one species. Asian *Persea* (subg. *Machilus*) places with paleotropical *Alseodaphne*, *Apollonias*, *Dehaasia*, and *Phoebe*, while American *Persea* (subg. *Eriodaphne* and subg. *Persea*) constitutes a separate clade (Fig. 3), but the implication that *Persea* is not monophyletic has little support. Detailed morphological and molecular studies are needed to resolve relationships within this large group of ca. 400 species, most of which are found in tropical Asia.

Laureae. Close relationship among *Actinodaphne*, *Adenodaphne*, *Laurus*, *Lindera*, *Litsea*, *Neolitsea*, *Parasassafras*, and *Sassafras* has been recognized in almost all classifications of Lauraceae. All are dioecious and most have umbellate inflorescences subtended by involucral bracts. In this study, parsimony analyses place *Sassafras* between well-supported core Laureae and remaining genera (Fig. 3). However, membership in Laureae was found by minimum evolution analyses, and parsimony-based trees in which *Sassafras* lies sister to core Laureae

(Fig. 4) are just three steps longer. Introrsely positioned locelli in all staminal whorls support a place for *Sassafras* in Laureae. Elsewhere in Lauraceae introrsely positioned locelli are restricted to the outer two staminal whorls. Another potential synapomorphy is the dioecious breeding system shown by *Sassafras* and core Laureae. In Lauraceae, dioecy is otherwise only found in basally positioned *Hypodaphnis* (Fig. 1) and a distal clade of *Ocotea* s. str., *Endlicheria*, and *Rhodostemonodaphne* (Fig. 3). *Dodecadenia* and *Cinnadenia*, not herein, should also place in Laureae on the basis of dioecy and introrse locelli.

Umbellularia is usually placed in Laureae because of its umbellate involucrate inflorescences (e.g., van der Werff & Richter, 1996), but its flowers are bisexual and locelli of the innermost staminal whorl are extrorse, not introrse. A 16-bp repeat in *trn*L-*trn*F (Fig. 1) and ITS sequences (Figs. 3, 4) distance *Umbellularia* from Laureae and place it in the *Ocotea* complex (below).

Generic delimitation in Laureae is unsettled. *Litsea* alone accommodates ca. 400 of the approximately 700 species, and most generic limits are probably artificial (Rohwer, 1993a; Li & Christophel, 2000). As with the *Persea* group, detailed systematic studies are needed to resolve natural lineages in Laureae.

Cinnamomeae. All remaining genera were previously placed in Cinnamomeae (sensu Kostermans, 1957), albeit together with *Sassafras*, *Actinodaphne*, and *Neocinnamomum*. With these three genera excluded, Cinnamomeae is van der Werff and Richter's (1996) Perseeae without the *Persea* group. Cinnamomeae are thus a sizeable subset of the Perseeae–Laureae clade (Fig. 3), accommodating all of its major neotropical genera (e.g., *Aiouea*, *Aniba*, *Endlicheria*, *Licaria*, *Nectandra*, *Pleurothyrium*, and *Rhodostemonodaphne*) as well as widespread *Cinnamomum* and *Ocotea*.

Cinnamomeae share hemispherical cupules (rarely poorly developed) with Laureae and retain the thyrsoid non-involucrate inflorescences of the *Persea* group. Thus, uniquely derived features are not obvious. Bootstrap support for Cinnamomeae reaches only 52% in unconstrained parsimony analyses (Fig. 3), but raised to 86% by enforcing monophyly of a generic alliance centered around *Cinnamomum* (Fig. 4).

Cinnamomum *group.* The delimitation of *Cinnamomum* is based on its nine stamens with four-locular anthers and a fourth androecial whorl of well-developed staminodes provided with sagittate glandular apices. The present data nest two neotropical genera in *Cinnamomum*. Monotypic *Mocinnodaphne* was described to recognize a reduction in number of staminal whorls (Lorea-Hernandez, 1995), and *Aiouea* p.p. [*A. dubia* (HBK) Mez and *A. guianensis* Aubl. herein] differs mainly in locelli number, both characters of traditional generic value. The finding that *Ocotea ikonyokpe* van der Werff, a recently described species from Cameroon, is placed with *Cinnamomum* is surprising. However, a leaf fragment from the holotype sheet (*Thomas 10456*, MO) was extracted, amplified, and sequenced only with other species of *Ocotea*. Furthermore, ITS1 and ITS2 regions of *O. ikonyokpe* were amplified and sequenced separately. Neither section is identical with accessions of *Cinnamomum*, and both support a place with *Cinnamomum*. In Africa, *O. ikonyokpe* shares (sub)opposite leaves with East African *O. michelsonii* Robyns & Wilczek and *O. usambarensis* Engl. (not herein). All other African *Ocotea* have spirally arranged leaves (van der Werff, 1996). Interestingly, *O. ikonyokpe* associates with Asian *Cinnamomum* (mostly opposite-leaved) instead of mostly alternate-leaved neotropical *Cinnamomum* (Figs. 3, 4). The staminodes, relatively smaller than seen in *Cinnamomum*, and without sagittate apices (although glandular as in *Cinnamomum*), refer this Cameroon species to *Ocotea*, but leaf arrangement is perhaps an overlooked character here.

Neither molecular nor morphological synapomorphies readily appear for the *Cinnamomum* group (*Cinnamomum*, *Aiouea* p.p., *Mocinnodaphne*, and *Ocotea* p.p.), but enforcing monophyly adds only one step to parsimony-based trees (Fig. 4). Still, New and Old World species remain separate subclades in the constrained clade. This New World–Old World dichotomy is also evident in wood and bark anatomy (Richter, 1981), and can be deduced from traditional placement of neotropical *Cinnamomum* in *Phoebe* (of the *Persea* group above) until transferred by Kostermans (1961). With over 350 species distributed from (sub)tropical Asia to the Neotropics, one African member, and a few representatives in Australia and the Pacific Islands (primarily Fiji), the *Cinnamomum* group is speciose and widespread.

Ocotea *complex.* The remaining genera of Cinnamomeae form a strongly supported clade within which members of *Ocotea* are widely dispersed (Figs. 3, 4). Finding that *Umbellularia* places here, and not in the Laureae, clarifies conflicting indications from floral morphology and inflorescence structure (discussed under Laureae, above). Apart from a few Old World species of *Ocotea*, the com-

plex is restricted to the New World and accounts for most of the generic and species diversity of Lauraceae in the Neotropics.

Clades resolved within the *Ocotea* complex can be circumscribed in geographic, and sometimes morphological, terms. Basal branches in the complex are occupied by Old World species of *Ocotea*, North American *Umbellularia*, and primarily Central American species groups in *Nectandra* and *Ocotea*. Two derived clades are centered in South America. In the more speciose, *Nectandra* s. str. and *Pleurothyrium* lie sister to a dioecious clade comprised of *Endlicheria*, *Ocotea* s. str., and *Rhodostemonodaphne*. In the second and less speciose South America-centered clade, *Aniba*, an assortment of *Ocotea* species, and mostly mono- to oligotypic genera associate with *Licaria*.

Old World Ocotea. Outside of the Neotropics, *Ocotea* is represented by *O. foetens* (Aiton) Baill. in Macaronesia, 7 species in mainland Africa, and about 30 species in Madagascar. In this study, South African *O. bullata* (Burch.) E. Mey. and 2 species from Madagascar, *O. grayi* van der Werff and *O. malcomberi* van der Werff, constitute a strongly supported clade. *Ocotea foetens* is almost indistinguishable from *O. bullata*, but surprisingly its place with Old World *Ocotea* receives < 50% bootstrap support (Figs. 3, 4). Morphological characters distancing Old World *Ocotea* from New World relatives do not readily appear. In the present reconstruction Old World *Ocotea* lie sister to the New World component of the *Ocotea* complex.

Nectandra coriacea *species group*, Nectandra *s. str.*, *and* Pleurothyrium. Representatives of the *Nectandra coriacea* species group, *N. coriacea* (Sw.) Griseb., *N. salicifolia* (HBK) Nees, and *N. purpurea* (Ruiz & Pav.) Mez, place distant from a clade in which a broad morphological representation of *Nectandra* s. str. pairs with *Pleurothyrium* (Figs. 3, 4). Close relationship between *Nectandra* s. str. and *Pleurothyrium* was also indicated by *mat*K sequences (Rohwer, 2000). The two share relatively large rotate flowers with heavily papillose tepals and stamens, and relatively poorly developed staminodes. *Nectandra* s. str. and *Pleurothyrium* are centered in South America with ca. 100 and 40 species, respectively. The Central America-centered *Nectandra coriacea* species group (ca. 20 spp.) was perceived as accommodating the most primitive elements of *Nectandra*, retaining *Ocotea*-like non-scalariform venation, tepal and stamen non-papillosity, distinct filaments, and a well-developed fourth androecial whorl of staminodes with glandular apices (Rohwer, 1993b; Rohwer & Kubitzki, 1993). A relatively basal position in the *Ocotea* complex is suggested by ITS sequences (Figs. 3, 4).

Ocotea helicterifolia *species group*. The clade (Figs. 3, 4) comprised of *Ocotea botrantha* Rohwer, *O. helicterifolia* (Meisn.) Hemsl., and *O. heydeana* (Mez & Donn. Sm.) Bernardi represents a diverse but cohesive assemblage of species in *Ocotea*. Core members are characterized by hirsute leaves and twigs, bisexual flowers with partially papillose tepals, glabrous or weakly papillose anthers with four pollen sacs arranged in two superimposed pairs, and well-developed staminodes (van der Werff, 1999). Close relationship with non-hirsute species with this general floral structure, i.e., the *Ocotea heydeana* species group, and with the *Ocotea sinuata* species group, which differs by more heavily papillose tepals and anthers (here represented by *O. botrantha* Rohwer) was anticipated by Rohwer (1991). With the *Nectandra coriacea* species group, and *Umbellularia*, the *Ocotea helicterifolia* species group shares well-developed staminodes with glandular apices, while their papillose anthers and tepals suggest an affinity with *Nectandra* s. str. and *Pleurothyrium*. Their place in the present phylogeny is consistent with this intermediate morphology. The group is distributed throughout Central America and numbers around 30 species.

Ocotea *s. str.*, Endlicheria, *and* Rhodostemonodaphne. A clade comprised of all dioecious *Ocotea* sampled for this study and the only neotropical genera with this breeding system, *Endlicheria* and *Rhodostemonodaphne*, is found in all ITS-based trees. It receives low bootstrap support as a whole (55%), but much better (93%) support within the group above the branch separating *Endlicheria punctulata* (Mez) C. K. Allen and *Ocotea pauciflora* (Nees) Mez from the rest (Fig. 3). As seen in Figure 4, *E. punctulata* and *O. pauciflora* appear to diverge early, shortening the branch supporting the entire clade, but this effect could also be obtained by reversals to ancestral molecular states along the branch uniting these two species.

Taxa placed here include the type species of *Ocotea*, *O. guianensis* Aubl., and represent several species groups recognizable among dioecious *Ocotea* (e.g., Rohwer, 1986). Since representatives of *Endlicheria* and *Rhodostemonodaphne* also sample a wide range of morphological variation within these moderately sized but heterogeneous genera, all approximately 300 dioecious species of the *Ocotea* complex should place here. In this clade generic limits based on locelli arrangement and number are not supported. The *Ocotea* species form a

paraphyletic assemblage within which *Rhodostemonodaphne* and *Endlicheria* are nested (Figs. 3, 4). *Rhodostemonodaphne* has four-locular anthers like *Ocotea*, but the locelli are arranged in a shallow arc or horizontal row rather than superimposed pairs, at least in the outer stamens. *Endlicheria* is two-locular, but with *E. punctulata* paired with *O. pauciflora*, and other species placed with *Rhodostemonodaphne* (Figs. 3, 4), it is at least di-phyletic.

Licaria *group.* The branch uniting *Dicypellium*, *Kubitzkia*, *Licaria*, *Paraia*, *Urbanodendron*, and two species of *Ocotea* receives 93% bootstrap support (Fig. 3) and seems to be a natural alliance. *Gamanthera* and *Phyllostemonodaphne* (neither herein) associate with the *Licaria* group on morphological grounds. All these genera have cupules with double-rimmed (or more) margins. Given these unusual cupules, a generic alliance centered around *Licaria* has been informally recognized in recent morphological systems (Kostermans, 1957; Rohwer, 1993a). *Ocotea quixos* (Lam.) Kosterm. and *O. veraguensis* (Meisn.) Mez represent the *Ocotea dendrodaphne* species group, a group of 8 species remarkable in *Ocotea* for their ligulate stamens and double-rimmed cupules. A place in the *Licaria* group is thus not surprising. Their distance from the rest of *Ocotea* has been acknowledged by subgeneric (e.g., Mez, 1889; Rohwer, 1986) and even generic status; e.g., *Sassafridium* (Meissner, 1864). Hutchinson (1964) even placed *O. veraguensis* (as *Sassafridium*) in a monotypic tribe because he interpreted the locelli in the third staminal whorl to be introrse, a condition only known in Laureae. In fact the locelli are latrorse-introrse, as also found in *Dicypellium* and *Urbanodendron*, both of the *Licaria* group. Elsewhere in *Ocotea*, double-rimmed cupules are found in a few dioecious species, e.g., *O. cujumary* Mart. and *O. floribunda* (Sw.) Mez. It is unlikely that these species will assign to the *Licaria* group since their morphologically close relatives, *O. guianensis* Aubl. and *O. percoriacea* (Meisn.) Kosterm., respectively, are firmly seated in the dioecious clade discussed above.

Remaining taxa place near the *Licaria* group without strong support (Fig. 3). *Ocotea rhynchophylla* (Meisn.) Mez and *O. odorifera* (Vell.) Rohwer represent species groups that Rohwer (1986) considered intermediate between the *O. dendrodaphne* species group (including *O. quixos* and *O. veraguensis* herein) and the rest of *Ocotea*. Their position near the *Licaria* group is consistent with Rohwer's interpretation. *Aniba*, too, has been associated with *Licaria* (e.g., Kubitzki, 1982), but has simple-rimmed or rarely weakly double-rimmed cupules. *Ocotea insularis* (Meisn.) Mez and *Aiouea costaricensis* (Mez) Kosterm. are united with 100% bootstrap support (Figs. 3, 4). The two differ only in the number of locules per anther, again illustrating the weakness of this character in generic delimitation (van der Werff, 1984). Curiously, as noted by van der Werff (1988) and Rohwer et al. (1991), like *A. costaricensis*, other *Aiouea* species with closer ties to *Ocotea* are Central American (e.g., *A. lundelliana* C. K. Allen, not herein), while South American *Aiouea* associate with *Cinnamomum* (e.g., *A. dubia* and *A. guianensis*, Fig. 3).

Biogeography

Genera and clades in Lauraceae sort into two main geographic groups (Figs. 2, 4). *Hypodaphnis*, the Cryptocaryeae, *Cassytha*, and the *Chlorocardium–Mezilaurus* clade as seen in Figure 1 are predominantly or entirely southern hemispheric, while *Caryodaphnopsis*, *Neocinnamomum*, the *Persea* group, the *Cinnamomum* group, and Laureae are either Asian or have amphi-Pacific distributions (Figs. 2, 4). The distributions of these two main groups are consistent with Gondwanan and Laurasian histories, respectively, but the geographic distribution of the *Ocotea* complex is not as easily categorized. This diverse clade is mostly neotropical with a Macaronesia–Afro-Malagasy element added by approximately 40 Old World species of *Ocotea*. Whether ancestors of the *Ocotea* complex migrated into South America via Africa (Raven & Axelrod, 1974) or via North America (Rohwer, 1986; Taylor, 1988; Rohwer & Kubitzki, 1993; Rohwer, 2000) is not evident from the topology alone.

Paleogeographic reconstructions of West Gondwana breakup estimate that direct land connections between Africa and South America were lost by the Upper Mid-Cretaceous, ca. 90 Mya (Sclater et al., 1977; Scotese et al., 1988; Parrish, 1993). Therefore, if the trans-Atlantic disjunction in the *Ocotea* complex can be attributed to West Gondwanan breakup, it would have to be at least 90 Mya old. This biogeographic scenario was simulated on the parsimony-based ITS topology by fixing the New World–Old World divergence (node e in Fig. 5a and Table 3a) at 90 My and using the resulting rate of molecular evolution to estimate divergence times for other nodes. This calibration yields a rate of 0.000275 substitutions per site per million years, a rate that halves the lowest ever reported for ITS (Suh et al., 1993), and which places the divergence of the *Chlorocardium–Mezilaurus* clade (Fig. 3; and represented by *Sextonia pubescens* van der Werff in Fig. 5a) from the Perseeae–Laureae clade (includes

the *Ocotea* complex) at 354 Mya (node a in Fig. 5a and Table 3a). This Devonian age precedes earliest undisputed angiosperm fossils. Furthermore, if the equivalent node in the basal topology (node G in Fig. 5b and Table 3b) is fixed at 354 Mya and divergence times for earlier lineages calculated, the radiation of the family is placed in the Pre-Cambrian, ca. 682 Mya. Therefore, the neotropical–African disjunction shown by the *Ocotea* complex is likely to be much younger than West Gondwanan breakup, and some amount of dispersal must have been involved.

How did the *Ocotea* complex, and other members of the family, reach the New World? In both morphological and molecular attributes the *Chlorocardium–Mezilaurus* clade occupies a basal and isolated position in Lauraceae. It is also the only lineage of basal Lauraceae restricted to South America (reaching its northern limit in Costa Rica). These qualities argue for early isolation of the *Chlorocardium–Mezilaurus* clade in South America, presumably initiated by West Gondwana breakup. To assess this biogeographic scenario on other divergence times, substitution rates in the ITS and *rpl*16 data sets were re-calibrated using an Upper Mid-Cretaceous (90 Mya) separation of South America from Africa to date the divergence of the *Chlorocardium–Mezilaurus* clade from its sister group (node a in Fig. 5a and Table 3a, node G in Fig. 5b and Table 3b). With this second calibration, radiation of Lauraceae was estimated at 174 ± 32 Mya (node A in Fig. 5b and Table 3b), and radiation of the terminal Perseeae–Laureae clade was placed in the Eocene, 44 ± 7 Mya (node b in Fig. 5a and Table 3a). This estimate for Eocene radiation of the latter group is supported by the fossil record. The hemispherical cupules of the London Clay Flora (Reid & Chandler, 1933) are restricted to Laureae and Cinnamomeae of the Perseeae–Laureae clade. Well-preserved flowers with the general floral structure of genera in the *Persea* group and Cinnamomeae, but not other members of Lauraceae, have been described from Eocene deposits in North America (Taylor, 1988) and Late Eocene Baltic amber (Conwentz, 1886). Thus, our second calibration, that which credits South American isolation of the *Chlorocardium–Mezilaurus* clade to West Gondwanan breakup, estimates a realistic age for radiation of the family and complements fossil evidence of radiation of the Perseeae–Laureae group in Early Tertiary Laurasia. This congruence provides confidence that age estimates based on our second calibration are good approximations of actual divergence times.

BASAL LINEAGES

Raven and Axelrod (1974) situated the cradle of the angiosperms in West Gondwana, and its proximity to Laurasia was pivotal to the Mid-Cretaceous presence of angiosperm pollen in the South Laurasia Province (sensu Brenner, 1976). Any explanation of the biogeographic history of Lauraceae also requires early migratory routes between Laurasia and West Gondwana. Southern hemispheric *Hypodaphnis*, Cryptocaryeae, *Cassytha*, and the *Chlorocardium–Mezilaurus* clade indicate Gondwanan history, but *Caryodaphnopsis* and *Neocinnamomum* appear to be Laurasian.

Caryodaphnopsis is disjunct between tropical America and tropical Asia, while *Neocinnamomum* is known only from tropical Asia. They represent the only early lineages in Lauraceae that are present in Asia but are not also known to occur in Africa, Madagascar, and Australia, in contrast to widespread genera in Cryptocaryeae and *Cassytha*. The fossil record suggests that both *Caryodaphnopsis* and *Neocinnamomum* have an ancient Laurasian history. The fossil wood taxon *Caryodaphnopsoxylon richteri* Gottwald (1992) places the unique xylem anatomy of *Caryodaphnopsis* in Late Eocene Germany. The fossil flower *Neusenia tetrasporangiata* Eklund from Late Cretaceous North America compares favorably with *Neocinnamomum*, and flowers and fruits from the same locality can be compared to *Caryodaphnopsis* (e.g., Eklund, 2000). Although the affinities of the latter fossils cannot be unambiguously assigned, the implied antiquity of *Caryodaphnopsis* and *Neocinnamomum* is consistent with their relatively basal positions in Lauraceae. Most likely, these modern genera are descendants of the Cretaceous Laurasian flora of Lauraceae documented by the fossil genera *Mauldinia* (Drinnan et al., 1990; Herendeen et al., 1994, 1999; Eklund & Kvaček, 1998) and *Perseanthus* (Herendeen et al., 1994).

The timing of events that resulted in the modern distribution of *Caryodaphnopsis* can further elucidate its biogeographic history. A relictual presence in South America would imply a continuous paleodistribution from South America to Eurasia. However, such continental configuration also provides direct connections between South America and other Gondwanan terrains (reviewed in Hallam, 1994), and preferential extinction in these Southern landmasses would be necessary to explain the modern disjunction. Alternatively, the *rpl*16 data set estimates a relatively recent separation (44 ± 14 Mya) of South American *C. tomentosa* van der Werff from Asian *C. bilocellata* van der Werff (node F in Fig.

5b and Table 3b). This would rule out a relictual presence in South America and is consistent with the view that disjunct distributions between tropical Asia and tropical America are derived from ancestral boreotropical ranges disrupted by Late Eocene climatic cooling (e.g., Wolfe, 1975; Tiffney, 1985a, b; Zhengyi, 1983). Moreover, this calibration implies that isolation of *Caryodaphnopsis* from the rest of the family can be staged in the Early Cretaceous about 140 Mya (node E in Fig. 5b and Table 3b). Increasing separation of Laurasia from Gondwana, a salient feature of Early Cretaceous paleogeography (reviewed in Hallam, 1994), would have disrupted trans-Tethyan ancestral ranges and precipitated the next biogeographic phase in Lauraceae, i.e., radiation on increasingly distant Laurasian and Gondwanan landmasses.

Accordingly, in the Northern Hemisphere, the Mid-Cretaceous fossil taxa, and the direct ancestors of *Caryodaphnopsis* and *Neocinnamomum*, would have spread throughout southern Laurasia until decreasing temperatures and the opening of the north Atlantic constricted their descendants to tropical Asia and America. To the south, truly pantropical genera and clades would have attained their widespread distribution, with seafloor spreading in the South Atlantic and Indian Oceans leading to increased regional endemicity. These continental reconfigurations appear to be reflected in the distribution of Southern Hemispheric genera.

Of pantropical genera, *Beilschmiedia* and *Cryptocarya* are the most widespread. The genetic distance-based age estimations indicate that these genera diverged from their most recent common ancestor about 90 ± 20 Mya (node D in Fig. 5b and Table 3b). Variance around these age estimations argues for direct migration throughout Gondwana, and a widespread pre-drift distribution for both *Beilschmiedia* and *Cryptocarya*. The presence of both genera in continental Asia may be due to the rafting of the Indian subcontinent and other Gondwanan fragments to the Asian plate. Later accretions of Gondwanan fragments with the Asian plate and Miocene island hopping across the Indo-Malayan region may also have been involved. The pantropical distribution of *Cassytha* is mostly due to one widespread species, *C. filiformis* L.; all other approximately 20 species are restricted to the Old World and show high regional endemism in Australia (Weber, 1981). Although the Southern Hemispheric distribution centered in the Old World favors a predominantly East Gondwanan history for *Cassytha*, the possibility of a Laurasian history followed by radiation into its associated xeric habitat cannot be discounted. All other Southern Hemispheric genera have narrower ranges.

Hypodaphnis may be relictual in Central Africa since its ancestors apparently diverged from the rest of the family when direct migration between Gondwana and Laurasia was possible (node A in Fig. 5b and Table 3a). *Eusideroxylon* ranges from Borneo to Sumatra, and *Potoxylon* is endemic to Borneo. With their placement in predominantly Southern Hemispheric Cryptocaryeae, it is possible to regard them as Gondwanan relicts as well. However, their separation from the rest of the tribe is dated at about 120 Mya (node C, Fig. 5b), an age that permits early migration into Laurasia, as envisioned for *Caryodaphnopsis* above. Further, *Trianthera eusideroxylon* Conwentz, an amber-embedded flower from the Eocene-Oligocene boundary of the Baltic area (Conwentz, 1886) compares remarkably well with *Eusideroxylon* and adds to the possibility of a Gondwanan–Laurasian dichotomy in Cryptocaryeae. Upper Cretaceous appearance of the boreotropical *Aquilapollenites* in Borneo (Wolfe, 1975; and references therein), and the composite geological nature of the Indo-Malayan region (Burrett et al., 1991; Michaux, 1991; and references therein), are also consistent with Laurasian history for *Eusideroxylon* and *Potoxylon*.

All other members of Cryptocaryeae and their allies are restricted to, or best represented in, austral parts of the Old World, i.e., East Gondwanan and derived terrains. At the other end of the former southern continent, the *Chlorocardium–Mezilaurus* clade is restricted to South America. Thus, among these Southern Hemispheric genera and clades, more basal groups are either widespread or relictual, and more derived groups are restricted to Eastern or Western Gondwanan fragments, consistent with the progressive dismantling of Gondwana.

The Perseeae–Laureae Clade

Routes to Laurasia

The Perseeae–Laureae clade diverged from its sister group, the *Chlorocardium–Mezilaurus* clade, since the Upper Mid-Cretaceous, but until fossil members appeared in Eocene Laurasia its biogeographic history is a mystery. Three alternative scenarios are conceivable. In a vicariant vein, consider a West Gondwanan common ancestor for the Perseeae–Laureae clade and its sister group, with tectonic activity isolating direct ancestors of the *Chlorocardium–Mezilaurus* clade on South America while stranding those of the Perseeae–Laureae clade on Africa. Ancestors of the Perseeae–Laureae clade then migrate to Laurasia via North Africa.

Alternatively, the northern route of the Perseeae–Laureae clade could have included a South American phase with subsequent migration to North America and Eurasia. Precursors of the Greater Antilles spanned the Panamanian Isthmus as an island chain in the Mid-Cretaceous (Pindell et al., 1988) providing a stepping-stone migratory route out of South America. Yet a third possibility is that the common ancestor of the Perseeae–Laureae and *Chlorocardium–Mezilaurus* clades was Laurasian and shared the former northern continent with *Caryodaphnopsis* and *Neocinnamomum*. This scenario would require that the *Chlorocardium–Mezilaurus* clade migrated into South America via the Mid-Cretaceous stepping-stone route provided by proto-Greater Antilles. Evidence of an early faunal and floristic exchange across this region has accumulated (e.g., Cadle, 1985; Estes & Baez, 1985; Graham, 1995; Burnham & Graham, 1999), but the taxa involved are usually distributed throughout northern Central America and South America, while the *Chlorocardium–Mezilaurus* clade only reaches Costa Rica to the north.

Without an unambiguous Cretaceous fossil record for the Perseeae–Laureae clade, we favor the first or African scenario. North Africa is today part of the largest desert system in the world, but was covered by tropical forest well into the Miocene (Axelrod & Raven, 1978). During the Tertiary, Africa moved progressively northward, and migration to Eurasia across the narrowing Tethys would have become increasingly feasible. The South American scenario invokes a circuitous route to Eurasia and fails to explain why no members of the Perseeae–Laureae clade remain relictual in South America. An early Laurasian history would have to account for the absence of the *Chlorocardium–Mezilaurus* clade in Asia.

Routes to the New World I: *Persea* Group, *Cinnamomum* Group, and Laureae

The *Persea* group is most diverse in Asia, with only ca. 80 of its approximately 400 species found in the New World. These occur mainly in montane forests in Central and South America and range from Mexico to Chile, reaching the Atlantic coastal forests in southeastern Brazil. In the Old World, two taxa are present in the Canary Islands. *Apollonias barbujana* (Cav.) Bornm. is placed in an unresolved Asian clade with *Alseodaphne, Dehaasia, Persea* subg. *Machilus*, and *Phoebe* (Figs. 3, 4). The other Canary Island endemic, *Persea indica* (L.) Spreng., was not sampled here. Pending better resolution within the *Persea* group, current age estimates suggest that its Asian and American members diverged around the Eocene–Oligocene boundary, ca. 32 Mya (node z in Fig. 5a and Table 3a).

The distribution and representation of the *Cinnamomum* group in the Neotropics and Asia is almost identical to that of the *Persea* group. Whether New and Old World clades in the *Cinnamomum* group constitute a monophyletic group is not yet clear. From age estimates obtained by enforcing monophyly (Fig. 5a), the assumed common ancestral gene pool divided around the Eocene–Oligocene boundary, a divergence time coincident with that calculated for the *Persea* group. These similarities in the tropical amphi-Pacific disjunctions in the *Cinnamomum* and *Persea* groups suggest commonality. Disruption of boreotropical ranges by climatic cooling around the Eocene–Oligocene boundary (Wolfe, 1975; Tiffney, 1985a, b; Zhengyi, 1983) would be consistent with the present age estimates. Already tenuous biotic links across the North Atlantic and/or Pacific were severed at this time, and divided *Cinnamomum* and *Persea* groups receded to warmer paleolatitudes in Asia and the Americas.

Like the *Cinnamomum* and *Persea* groups, Laureae are most diverse in Asia with a smaller range and representation in the New World. *Lindera, Litsea*, and *Sassafras* reach the Americas, but of these only *Litsea* ranges south of temperate North America, to Costa Rica. The unsettled generic delimitation of *Litsea* and *Lindera* cautions against assessment of their disjunctions from morphology, but monophyly of *Sassafras* is well supported here (Figs. 3, 4), providing an opportunity to assess the classic North America–Eastern Asia disjunction first brought into scientific focus by Asa Gray (Boufford & Spongberg, 1983). In *Sassafras*, divergence of Asian *S. tzumu* (Hemsl.) Hemsl. from North American *S. albidum* (Nutt.) Nees was estimated at about 12 Mya (node x in Fig. 5a and Table 3a). This northern temperate disjunction is much younger than the ± 30 Mya old tropical amphi-Pacific disjunctions shown by *Cinnamomum* and *Persea* groups. While climatic cooling in the Eocene and Oligocene (Wolfe, 1975; Hallam, 1994) restricted tropical taxa to lower paleolatitudes, for temperate taxa intercontinental connections across northern latitudes would have been possible until much later in the Tertiary. The estimated divergence time separating Asian from North American *Sassafras* coincides well with opening of the Bering Strait, and supports Wolfe and Leopold's (1967) view that Mid-Miocene loss of the Bering land bridge is the most likely cause of north-temperate disjunctions between North America and Asia.

ROUTES TO THE NEW WORLD II: THE *OCOTEA* COMPLEX

The *Ocotea* complex accommodates most of the taxonomic diversity of neotropical Lauraceae. In the Old World the complex is weakly represented in Macaronesia, Africa, and Madagascar. Any trans-Atlantic disjunction produced by West Gondwanan vicariance was discounted (above) and instead the disjunction dates to around the Oligocene–Miocene boundary, ca. 23 ± 5 Mya (node e in Fig. 5a and Table 3a). The estimated Eocene–Oligocene age (node d in Fig. 5a and Table 3a) of the *Ocotea* complex implies an origin concurrent with the southward movement of megathermal forests (Wolfe, 1975; Hallam, 1994), and its derived position relative to the previous clades indicates boreotropical ancestry. Unlike previous Laurasian taxa, the *Ocotea* complex is absent in Asia. While *Persea* and *Cinnamomum* groups appear as lower montane taxa in the Neotropics, the *Ocotea* complex is especially diverse in the lowlands of South America. Given these differences in distribution and a relatively recent trans-Atlantic disjunction, their biogeographic history may be quite different from that of the other boreotropical lineages. In this regard xeric tolerances shown by African *Ocotea*, Californian *Umbellularia*, and the Central America-centered *Nectandra coriacea* group may be significant. These taxa place basally in the complex, and their sclerophyllous habit, unusual for Lauraceae, adds to taxa that link the Madrean–Tethyan sclerophyllous flora discussed by Axelrod (1975). This broad-leaved flora ranged along the Tethyan coast from North America to southeastern Eurasia and North Africa, and existed relatively continuously since the Late Eocene, only disrupted by increased climatic cooling and drying at the end of the Oligocene (Axelrod, 1975). The 23 ± 5 Mya estimate of the trans-Atlantic disjunction in the *Ocotea* complex is consistent with that expected for taxa with ancestral Madrean–Tethyan ranges (e.g., Fritsch, 1996). Great disparity in species diversity on the two sides of the Atlantic may be attributed to disproportionate opportunities for speciation and differentiation. In the Neogene, continental Africa moves progressively northward into a drier and cooler climate (Hallam, 1994), while tectonic uplift in the Panamanian isthmus (Pindell et al., 1988) provides the Madrean flora of southeastern North America with opportunities for stepping-stone dispersal into South America.

Separation of the Central America-centered *Ocotea helicterifolia* species group from its speciose South America-centered sister group (Fig. 4) approximately 20 Mya (node f in Fig.5a and Table 3a) argues for arrival of the *Ocotea* complex in South America prior to Pliocene closure of the Panamanian isthmus. As the timing of the separation coincides with increased uplift of the northern Andes in the early Miocene, it is conceivable that Andean orogeny divided the ancestral range. Further, since lowland genera of the *Ocotea* complex place in either South- or Central America-centered clades (Fig. 4), Andean orogeny appears to maintain generic endemism while allowing lower montane *Cinnamomum* and *Persea* groups to range widely. Exceptionally, South America-centered clades range throughout Central America with widespread species, e.g., *N. cuspidata* Nees of *Nectandra* s. str. (Fig. 4), and vice versa, e.g., *N. purpurea* of the *Nectandra coriacea* species group (Fig. 4). Although these may be secondary range expansions of indigenous South and Central American taxa (e.g., Rohwer & Kubitzki, 1993), they indicate the underlying complexity of biogeographic patterns.

The biogeographic history of Lauraceae outlined here shares much with that proposed by Doyle and Le Thomas (1997) for Annonaceae. As in that diverse magnoliid family, three main phases are recognizable. Early radiation of both families apparently occurred when migration between Gondwana and Laurasia was possible. Next, diversification throughout the Cretaceous produced lauraceous Cryptocaryeae, perhaps *Cassytha*, and the *Chlorocardium–Mezilaurus* clade on Gondwana, with *Caryodaphnopsis* and its allies on Laurasia. In Annonaceae, *Anaxagorea* appears to be the counterpart of *Caryodaphnopsis*. In both families, renewed contact between Gondwanan and Laurasian fragments in the Early Tertiary resulted in a second radiation on Laurasian terrains. In Lauraceae, this boreotropical phase produced the Perseeae–Laureae clade, but unlike Annonaceae, its descendants did not only recede to the Asian tropics with climatic cooling. Three of the four major lineages of Lauraceae evolved during this period, migrated to the Neotropics, and one of these, the *Ocotea* complex, underwent a major radiation in the New World. This latter radiation has few parallels in neotropical phytogeography. There are indications that some lineages in the Leguminosae (Lavin & Luckow, 1993) and Melastomataceae (Renner & Meyer, in press) are derived from boreotropical ancestors, and Krutzsch (1989) listed possible examples from Bombacaceae, Olacaceae, and Symplocaceae. The emerging prospect of a larger contingent of Laurasian elements in the lowland Neotropics than previously recognized can be assessed when phylogenies of more tropical taxa become available.

Outlook: Toward a Phylogenetic Arrangement of Lauraceae

The utility of morphological classification that expresses evolutionary history and relationships with support from molecular data is obvious. For Lauraceae, appropriate characters to be employed in such a scheme are elusive. Among traditional morphological characters, the number of locules per anther attains generic and even supra-generic importance in early schemes (Nees, 1836; Meissner, 1864; Bentham, 1880; Pax, 1889; Mez, 1889; Kostermans, 1957). Several examples of the weakness of this character have been identified and are confirmed by the present molecular data. Other characters do not fare much better. The use of umbellate involucrate inflorescences to circumscribe Laureae is a salient feature of most classification schemes (e.g., Rohwer, 1993a; van der Werff & Richter, 1996), but this syndrome clearly evolved independently in *Umbellularia*. Similarly, dioecy appears three times on the phylogeny herein, in *Hypodaphnis*, in Laureae, and again in the *Ocotea* complex. Further, Kostermans' (1957) system stresses degree of cupule development, but both extremes are found in Cryptocaryeae, and the noncupulate condition of *Beilschmiedia* and *Endiandra* therein reappears in the distantly related *Persea* group. Equally problematical, morphological synapomorphies are not readily noted for several clades that receive strong molecular support. In the case of *Cassytha* and *Neocinnamomum* long branch attraction can be held responsible, but on closer examination genera of the *Chlorocardium–Mezilaurus* clade can be allied by a mosaic of characters.

Although major clades identified by molecular characters do not yield easily to morphological interpretation, a consensus over major generic groupings in Lauraceae appears to be within reach. Cryptocaryeae as circumscribed by van der Werff and Richter (1996), but probably without *Hypodaphnis*, are now supported by anatomy (Richter, 1981), embryology (Heo et al., 1998), and molecular data (Rohwer, 2000; herein). Considerable molecular support exists for the *Chlorocardium–Mezilaurus* clade, a group that is unique from both biogeographic and morphological perspectives. Further consensus concerns a large clade comprised of most remaining genera in Lauraceae that has been found by this and previous molecular data (Rohwer, 2000) and supported by anatomy (Richter, 1981). This group includes all genera placed in the tribes Perseeae and Laureae by van der Werff and Richter (1996), but its subdivision warrants re-examination. Toward this, Perseeae could be more narrowly circumscribed to accommodate just the *Persea* group, and *Umbellularia* removed from Laureae to Cinnamomeae. The latter also includes all remaining genera of van der Werff and Richter's Perseeae. Several smaller, although morphologically distinctive, taxa are not clearly placed, particularly in the case of *Cassytha*, to a lesser extent for *Caryodaphnopsis* and *Neocinnamomum*, and perhaps *Hypodaphnis*. At a lower taxonomic level, increased sampling is necessary for a thorough reconsideration of current generic concepts among Lauraceae. In this study, only the *Ocotea* complex has been representatively sampled; seemingly natural groups of genera and parts of larger genera have been identified within this complex. Similarly, increased sampling in other main clades identified here will improve our understanding of relationships among Lauraceae.

Literature Cited

Asmussen, C. 1999. Toward a chloroplast DNA phylogeny of the tribe Geonomeae (Palmae). Mem. New York Bot. Gard. 83: 121–129.

Axelrod, D. 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. Ann. Missouri Bot. Gard. 62: 280–334.

——— & P. Raven. 1978. Late Cretaceous and Tertiary vegetation history of Africa. Pp. 77–130 *in* M. Werger (editor), Biogeography and Ecology of Southern Africa. Junk, The Hague.

Bambacioni-Mezzetti, V. 1941. Ricerche morfologiche sulle Lauracee, embriologia della *Umbellularia californica* Nutt. e del *Laurus canariensis* Webb. Ann. Bot. (Rome) 22: 99–119.

Bentham, G. 1880. Laurinae. Pp. 146–165 *in* G. Bentham & J. Hooker (editors), Genera Plantarum 3. L. Reeve, London.

Blattner, F. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. Biotechniques 29: 1180–1186.

Boufford, D. & J. Spongberg. 1983. Eastern Asian-eastern North American phytogeographical relationships—A history from the time of Linnaeus to the twentieth century. Ann. Missouri Bot. Gard. 70: 423–439.

Brenner, G. 1976. Middle Cretaceous floral provinces and early migrations of angiosperms. Pp. 23–47 *in* C. Beck (editor), Origins and Early Evolution of Angiosperms. Columbia Univ. Press, New York.

Burnham, R. & A. Graham. 1999. The history of neotropical vegetation: New developments and status. Ann. Missouri Bot. Gard. 86: 546–589.

Burrett, C., N. Duhig, R. Berry & R. Varne. 1991. Asian and south-western Pacific continental terranes derived from Gondwana, and their biogeographic significance. Austral. Syst. Bot. 4: 13–24.

Cadle, J. 1985. The neotropical Colubrid snake fauna (Serpentes: Colubridae): Lineage components and biogeography. Syst. Zool. 34: 1–20.

Conwentz, H. 1886. Die Flora des Bernsteins, Vol. 2. Die Angiosperms des Bernsteins. Willhelm Engelmann, Danzig.

Doyle, J. & A. Le Thomas. 1997. Phylogeny and geo-

graphic history of Annonaceae. Géogr. Phys. Quatern. 51: 353–361.

——— & P. Endress. 2000. Morphological phylogenetic analysis of basal angiosperms: Comparison and combination with molecular data. Int. J. Pl. Sci. 161(Suppl.): S121–S153.

Drinnan, A., P. Crane, E. Friis & K. Pedersen. 1990. Lauraceous flowers form the Potomac Group (Mid-Cretaceous) of Eastern North America. Bot. Gaz. 151: 370–384.

Eklund, H. 2000. Lauraceous flowers from the Late Cretaceous of North Carolina, U.S.A. Bot. J. Linn. Soc. 132: 397–428.

——— & J. Kvaček. 1998. Lauraceous inflorescences and flowers from the Cenomanian of Bohemia (Czech Republic, Central Europe). Int. J. Pl. Sci. 159: 668–686.

Estes, R. & A. Baez. 1985. Herpetofaunas of North and South America during the Late Cretaceous and Cenozoic: Evidence for interchange? Pp. 139–197 *in* F. Stehli & S. Webb (editors), The Great American Biotic Interchange. Plenum Press, New York.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.

———. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Fritsch, P. 1996. Isozyme analysis of intercontinental disjuncts within *Styrax* (Styracaceae): Implications for the Madrean-Tethyan hypothesis. Amer. J. Bot. 83: 342–355.

Gentry, A. 1982. Neotropical floristic diversity: Phytogeographical connections between Central and South America, Pleistocene climate fluctuations, or an accident of the Andean orogeny? Ann. Missouri Bot. Gard. 69: 557–593.

———. 1988. Changes in plant community diversity and floristic composition on environmental and geographical gradients. Ann. Missouri Bot. Gard. 75: 1–34.

Goldblatt, P. (Editor). 1993. Biological Relationships Between Africa and South America. Yale Univ. Press, New Haven.

Gottwald, H. 1992. Hölzer aus marinen Sanden des oberen Eozän von Helmstedt (Niedersachsen). Palaeontographica, Abt. B. Paläophytol. 225: 27–103.

Graham, A. 1995. Development of affinities between Mexican/Central American and northern South American lowland and lower montane vegetation during the Tertiary. Pp. 11–22 *in* S. Churchill, H. Balslev, E. Forero & J. Luteyn (editors), Biodiversity and Conservation of Neotropical Montane Forests. The New York Botanical Garden, New York.

Hallam, A. 1994. An Outline of Phanerozoic Biogeography. Oxford Univ. Press, New York.

Hasegawa, M., H. Kishino & T. Yano. 1985. Dating the human-ape splitting by a molecular clock. J. Molec. Evol. 22: 160–174.

Heo, K. & H. Tobe. 1995. Embryology and relationships of *Gyrocarpus* and *Hernandia* (Hernandiaceae). J. Pl. Res. 108: 327–341.

———, H. van der Werff & H. Tobe. 1998. Embryology and relationships of Lauraceae (Laurales). Bot. J. Linn. Soc. 126: 295–322.

Herendeen, P., W. Crepet & K. Nixon. 1994. Fossil flowers and pollen of Lauraceae from the Upper Cretaceous of New Jersey. Pl. Syst. Evol. 189: 29–40.

———, S. Magallon-Puebla, R. Lupia, R. Crane & J. Kobylinska. 1999. A preliminary conspectus of the Allon flora of the late Cretaceous (Late Santonian) of Central Georgia, U.S.A. Ann. Missouri Bot. Gard. 86: 407–471.

Hillis, D. & J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Bot. 42: 182–192.

Hutchinson, J. 1964. The Genera of Flowering Plants (Dicotyledonae), Vol. 1. Clarendon Press, London.

Hyland, B. 1989. A revision of Lauraceae in Australia (excluding *Cassytha*). Austral. Syst. Bot. 2: 135–267.

Kostermans, A. 1957. Lauraceae. Reinwardtia 4: 193–256.

———. 1961. The New World species of *Cinnamomum* Trew (Lauraceae). Reinwardtia 6: 17–24.

———. 1974a. A monograph of the genus *Neocinnamomum* Liou Ho. Reinwardtia 9: 85–96.

———. 1974b. A monograph of *Caryodaphnopsis* A. Shaw. Reinwardtia 9: 123–137.

———. 1993. Notes on *Triadodaphne* Kosterm. (Lauraceae). Rheedea 3: 129–131.

———, H. Pinkley & W. Stern. 1969. A new Amazonian arrow poison: *Ocotea venenosa*. Bot. Mus. Leafl. 22: 241–252.

Krutzsch, W. 1989. Paleogeography and historical phytogeography (paleochorology) in the Neophyticum. Pl. Syst. Evol. 162: 5–61.

Kubitzki, K. 1982. Lauraceae: *Aniba*. Fl. Neotrop. Monogr. 31: 1–84.

Kuzoff, R., J. Sweere, D. Soltis, P. Soltis & E. Zimmer. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. Molec. Biol. Evol. 15: 251–263.

Lavin, M. & M. Luckow. 1993. Origins and relationships of tropical North America in the context of the boreotropics hypothesis. Amer. J. Bot. 80: 1–14.

Li, J. & D. Christophel. 2000. Systematic relationships within the *Litsea* complex (Lauraceae): A cladistic analysis on the basis of morphological and leaf cuticle data. Austral. Syst. Bot. 13: 1–13.

Lorea-Hernandez, F. 1995. *Mocinnodaphne*: Un género nuevo de la familia Lauraceae en la flora de México. Acta Bot. Mex. 32: 25–32.

Lyons-Weiler, J. & G. Hoelzer. 1997. Escaping the Felsenstein Zone by detecting long branches in phylogenetic data. Molec. Phylogenet. Evol. 8: 375–384.

Meissner, C. 1864. Lauraceae. Pp. 1–260 *in* A. de Candolle (editor), Prodromus Systematis Naturalis Regni Vegetablis. XV. Sumptibus Victoris Masson et Fillii, Paris.

Merwe, J. van der, A. van Wyk & P. Kok. 1988. *Dahlgrenodendron*, a remarkable new genus of Lauraceae from Natal and Pondoland. S. Afr. J. Bot. 54: 80–88.

Mez, C. 1889. Lauraceae Americanae. Jahrb. Königl. Bot. Gart. Berlin 5: 1–556.

Michaux, B. 1991. Distributional patterns and tectonic development in Indonesia: Wallace reinterpreted. Austral. Syst. Bot. 4: 25–36.

Nees, C. 1836. Systema Laurinarum. Sumtibus Veitii et Sociorum. Berlin.

Parrish, J. 1993. The paleogeography of the opening South Atlantic. Pp. 8–27 *in* W. George & R. Lavocat (editors), The Africa–South America Connection. Clarendon Press, Oxford.

Pax, F. 1889. Lauraceae. Pp. 106–126 *in* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien. Vol. III. Engelmann, Leipzig.

Pindell, J., S. Cande, W. Pitman, D. Rowley, J. Dewey, J.

LaBrecque & W. Haxby. 1988. A plate-kinematic framework for models of Caribbean evolution. Technophysics 155: 121–138.
Qiu, Y-L., J. Lee, F. Bernasconi-Quadroni, D. Soltis, P. Soltis, M. Zanis, E. Zimmer, Z. Chen, V. Savolainen & M. Chase. 1999. The earliest angiosperms: Evidence from mitochondrial, plastid, and nuclear genomes. Nature 402: 404–407.
Raven, P. & D. Axelrod. 1974. Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 61: 539–673.
Reid, E. & M. Chandler. 1933. The Flora of the London Clay. British Museum (Natural History), London.
Renner, S. 1999. Circumscription and phylogeny of the Laurales: Evidence from molecular and morphological data. Amer. J. Bot. 89: 1301–1315.
——— & A. Chanderbali. 2000. What is the relationship among Hernandiaceae, Lauraceae, and Monimiaceae, and why is this question so difficult to answer? Int. J. Pl. Sci. 161(Suppl.): S109–S119.
——— & K. Meyer. Melastomeae come full circle: Biogeographic reconstruction and molecular dating. Evolution (in press).
Richter, H. 1981. Anatomie des sekundären Xylems und der Rinde der Lauraceae. Sonderb. Naturwiss. Vereins Hamburg 5: 1–148.
Rohwer, J. 1986. Prodromus einer Monographie der Gattung *Ocotea* Aubl. (Lauraceae). Mitt. Inst. Allg. Bot. Hamburg 20: 3–278.
———. 1991. Borderline cases between *Ocotea*, *Nectandra* and *Phoebe* (Lauraceae): The marginal species of the *Ocotea helicterifolia*-group, including the *O. heydeana*-group. Bot. Jahrb. Syst. 112: 365–397.
———. 1993a. Lauraceae. Pp. 426–437 *in* K. Kubitzki, J. Rohwer & V. Bittrich (editors), The Families and Genera of Flowering Plants. Springer-Verlag, Berlin.
———. 1993b. *Nectandra*. Fl. Neotrop. Monogr. 60: 1–332.
———. 2000. Towards a phylogenetic classification of the Lauraceae: Evidence from *mat*K sequences. Syst. Bot. 25: 60–71.
——— & K. Kubitzki. 1993. Ecological differentiation in *Nectandra* (Lauraceae) and its historical implications. Bot. Acta 106: 88–99.
———, H. Richter & H. van der Werff. 1991. Two new genera of Neotropical Lauraceae and critical remarks on the generic delimitation. Ann. Missouri Bot. Gard. 78: 388–400.
Sang, T., D. Crawford & T. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Amer. J. Bot. 84: 1120–1136.
Sclater, J., S. Hellinger & C. Tapscott. 1977. The paleobathymetry of the Atlantic Ocean from the Jurassic to the present. J. Geol. 85: 509–952.
Scotese, C., L. Gahagan & R. Larsen. 1988. Plate tectonic reconstructions of the Cretaceous and Cenozoic ocean basins. Technophysics 155: 27–48.
Siddall, M. & M. Whiting. 1999. Long-branch abstractions. Cladistics 15: 9–24.
Suh, Y., L. Thein, H. Reeve & E. Zimmer. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. Amer. J. Bot. 80: 1042–1055.
Swofford, D. 1998. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4.0b4. Illinois Natural History Survey, Champaign, Illinois.
Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Molec. Biol. 17: 1105–1109.
Taylor, D. 1988. Eocene floral evidence of Lauraceae: Corroboration of the North American megafossil record. Amer. J. Bot. 75: 948–957.
Tiffney, B. 1985a. Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. J. Arnold Arbor. 66: 243–273.
———. 1985b. The Eocene North Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. J. Arnold Arbor. 66: 3–94.
Weber, J. 1981. A taxonomic revision of *Cassytha* (Lauraceae) in Australia. J. Adelaide Bot. Gard. 3: 187–262.
Werff, H. van der. 1984. Notes on neotropical Lauraceae. Ann. Missouri Bot. Gard. 71: 1180–1183.
———. 1988. Eight new species and one new combination of neotropical Lauraceae. Ann. Missouri Bot. Gard. 75: 402–419.
———. 1989. A new species of *Persea* (Lauraceae) from Surinam, with a discussion of its position within the genus. Ann. Missouri Bot. Gard. 76: 939–941.
———. 1991. A key to the genera of Lauraceae in the New World. Ann. Missouri Bot. Gard. 78: 377–387.
———. 1996. *Ocotea ikonyokpe*, a new species of *Ocotea* from Cameroon. Novon 6: 460–462.
———. 1997. *Sextonia*, a new genus of Lauraceae from South America. Novon 7: 436–439.
———. 1999. New taxa and combinations in the *Ocotea helicterifolia* (Lauraceae) species group. Novon 9: 571–583.
———. An annotated key to the genera of Lauraceae in the *Flora Malesiana* region. Blumea (in press).
——— & H. G. Richter. 1996. Toward an improved classification of Lauraceae. Ann. Missouri Bot. Gard. 83: 409–418.
White, T., T. Birns, S. Lee & J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–332 *in* M. Innis, D. Gelfand, J. Sninsky & T. White (editors), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.
Wolfe, J. 1975. Some aspects of plant geography of the Northern Hemisphere during the late Cretaceous and Tertiary. Ann. Missouri Bot. Gard. 62: 264–279.
——— & E. Leopold. 1967. Neogene and Early Quaternary vegetation of northwestern North America and northeastern Asia. Pp. 193–206 *in* D. Hopkins (editor), The Bering Landbridge. Stanford Univ. Press, Stanford.
Zhengyi, W. 1983. On the significance of Pacific intercontinental discontinuity. Ann. Missouri Bot. Gard. 70: 577–590.

THE PHYLOGENETIC RELATIONSHIPS OF *LEMBOCARPUS* AND *GOYAZIA* (GESNERIACEAE) BASED ON *ndh*F SEQUENCES[1]

James F. Smith[2]

ABSTRACT

The phylogenetic relationships of *Goyazia* and *Lembocarpus* are investigated based on cladistic analysis of *ndh*F sequences. Both genera are currently classified in the tribe Gloxinieae, but both are poorly known. Based on its floral morphology, the classification of *Goyazia* in Gloxinieae is not controversial. *Lembocarpus* may be placed in Gloxinieae, Episcieae, or Sinningieae. The acaulescent, tuberous nature of *Lembocarpus* limits the number of characters available for a morphological analysis and has made its classification and phylogenetic relationships difficult to resolve. Phylogenetic analyses of *ndh*F sequences place both genera in Gloxinieae. Although the affinities within the tribe are ambiguous for *Goyazia*, *Lembocarpus* is sister to *Capanea*. The addition of *Goyazia*, *Lembocarpus*, and an additional species of *Capanea* provide better resolution of relationships within Gloxinieae and Gesnerieae than had been obtained previously from parsimony analysis. A maximum likelihood analysis is largely congruent with the parsimony tree.

Key words: cladistics, Gesneriaceae, Gloxinieae, *Goyazia*, *Lembocarpus*, *ndh*F.

Classification and phylogenetic analyses within Gesneriaceae, particularly the neotropical subfamily Gesnerioideae, have received much attention, revealing numerous relationships within genera (Smith & Sytsma, 1994a, b, c; Smith, 1994; Kvist & Skog, 1988, 1989, 1993, 1996; Kvist, 1990), within tribes (Smith & Carroll, 1997; Smith & Atkinson, 1998; Smith, 2000a, b), and among tribes (Burtt & Wiehler, 1995; Smith, 1996, 2000c; Smith et al., 1997a, b). However, the classification and phylogenetic relationships of numerous genera have remained unexamined. Among these genera are *Lembocarpus*, endemic to Surinam and French Guiana, and *Goyazia* from Brazil.

Although collections of *Goyazia* are not common or well known and the plant is not in cultivation in North American or European gardens, the placement of this genus in Gloxinieae (Wiehler, 1983) has not been questioned. *Goyazia* is endemic to the Planalto of Brazil in Goiás and Mato Grosso provinces. The plant is a creeping saxicolous perennial with slender stems, scaly rhizomes, and small orbicular-ovate leaves. Flowers are borne singly in the leaf axils and are structurally the same as those of *Achimenes* Pers. and other members of Gloxinieae. Wiehler (1983) placed *Goyazia* in his Gloxinieae on the basis of its scaly rhizome, annular nectary, and corolla shape.

Unlike *Goyazia*, the placement of *Lembocarpus* within Gloxinieae (Wiehler, 1983) has drawn criticism. *Lembocarpus* is a poorly known, monotypic genus that was once in cultivation in North America, and apparently is no longer. The plant is an acaulescent tuberous perennial that produces a single leaf and inflorescence each season (Wiehler, 1983). The ovary is superior and the nectary is annular but nonfunctional (Wiehler, 1983). In his treatment of the Gesneriaceae of the Guianas, Leeuwenberg (1958) considered *Lembocarpus* to be similar to both the Guianan endemic *Rhoogeton* Leeuw. (tribe Episcieae), and *Gloxinia* L'Hér. (tribe Gloxinieae).

Wiehler (1983) was the first to place *Lembocarpus* in Gloxinieae on the basis of the annular nectary and tuberous habit. Additionally, Wiehler (1983) cited hybrids between *Lembocarpus* and *Sinningia* Nees (Gloxinieae sensu Wiehler, 1983) as additional support for *Lembocarpus* in Gloxinieae. Several *Sinningia* species have tubers and nearly superior ovaries, which added further support for

[1] Support for this project was provided by NSF grant DEB-9317775, a grant from the American Gloxinia and Gesneriad Society, and a grant from Boise State University Faculty Research Associates Program. I thank the following for providing leaf material: L. E. Skog and J. Boggan of the Smithsonian Institution, R. Dunn, D. Turley, R. Stewart, and M. Peixoto. I also thank M. Peixoto and A. Chautems for assistance in Brazil, and two anonymous reviewers for helpful comments.

[2] Department of Biology, Boise State University, 1910 University Drive, Boise, Idaho 83725, U.S.A. jsmith14@email.boisestate.edu

Table 1. Species sequenced in this study with GenBank submission numbers and voucher specimens. Letters in parentheses indicate herbaria where vouchers are deposited.

Species	Collection and voucher	GenBank number
Achimenes skinneri Lindl.	Cultivated (US)	U62177
Anodiscus xanthophyllus (Poepp.) Mansf.	Peru. *Dunn s.n.* (SRP)	AF040143
Bellonia sp.	Dominican Republic. *Evans s.n.* (SRP)	AF040144
Capanea affinis Fritsch	Colombia. Risaralda: Mpio. Santuario, Apr. 1998, *Amaya M. & Smith 393* (COL)	AF206201
C. grandiflora (Kunth) Decne. ex Planch.	Venezuela. Mérida: Sierra Nevada, Mucuy region, 26 Jan. 1989, *Smith 1199* (WIS)	AF040145
Diastema racemiferum Benth.	Cultivated. *Skog 7574* (US)	U62156
Eucodonia andrieuxii (DC.) Wiehler	Cultivated. *Dunn s. n.* (SRP)	AF040146
Gloxinia sylvatica (HBK) Kunth	Cultivated. *Dunn 9012051* (SRP)	U62157
Goyazia rupicola Taubert	Brazil. Goiás: 17 Jan. 1998, *Smith et al. 3722* (SRP)	AF257485
Heppiella ulmifolia (Kunth) Hanst.	Ecuador. Napo: near Baeza, 4 Apr. 1996, *Smith 3427* (SRP)	AF040147
Koellikeria erinoides (DC.) Mansf.	Brazil. *Dunn s.n.* (SRP)	AF013709
Kohleria spicata (Kunth) Oerst.	Cultivated. *Skog 7701* (US)	U62181
Lembocarpus amoenus Leeuw.	French Guiana. Haut Oyapock, Mt. St. Marcel, 21 Mar. 1976, *Sastre 4478* (US)	AF257486
Monopyle macrocarpa Benth.	Cultivated (US)	U62197
Moussonia strigosa (C. V. Morton)	Cultivated. *Dunn s.n.* (SRP)	AF040148
Niphaea oblonga Lindl.	Mexico. *Skog 7564* (US)	U62160
Pearcea sp.	Ecuador. Napo: near Baeza, 4 Apr. 1996, *Smith 3425* (SRP)	AF040149
P. hypocyrtiflora Regel	South America. Cultivated. *Smith 3943* (SRP)	AF040150
Phinaea albiflora Rusby	Cultivated (US)	AF040151
Sanango racemosum (Ruiz & Pav.) Barringer	Ecuador. Wiehler (GRF)	U62144
Smithiantha cinnabarina (Linden) Kuntze	Cultivated (US)	AF040152
Solenophora obliqua D. L. Denham & D. N. Gibson	Mexico. *Breedlove 71542* (CAS)	U62202
Gesnerieae		
Gesneria pedicellaris Alain	Cultivated. *Skog 7722* (US)	U62192
Gesneria christii Urban	Cultivated (US)	U62191
Rytidophyllum tomentosum (L.) Mart.	Cultivated. *Skog 5364* (US)	U62200
Rytidophyllum auriculatum Hook.	Cultivated (US)	U62199
Episcieae		
Alloplectus panamensis C. V. Morton	Panama. 28 Oct. 1993, *Skog et al. 7641* (US)	AF013685
Alloplectus sp.	Ecuador. Napo: San Rafael Falls, 4 Apr. 1996, *Smith 3418* (SRP)	AF013686
Alsobia dianthiflora (H. E. Moore & R. G. Wilson) Wiehler	Cultivated. *Skog 7969* (US)	AF013687
A. punctata (Lindl.) Wiehler	Mexico. Chiapas: Ocozocautla, winter 1991–1992, *Smith 3600* (SRP)	AF013688
Alsobia sp.	Cultivated (US)	AF013689
Alsobia sp.	Cultivated. *Smith 3599* (SRP)	AF013690
Chrysothemis friedrichsthaliana (Hanst.) H. E. Moore	Cultivated. *Skog 7992* (US)	AF013691
Cobananthus calochlamys (J. D. Sm.) Wiehler	Cultivated (US)	AF013692
Codonanthe elegans Wiehler	Belize. San Jose: *Skog 5699* (US)	U62178
Codonanthopsis peruviana Wiehler	Cultivated. *Turley s.n.* (SRP)	AF013693
Columnea ambigua (Urban) Morley	Puerto Rico. *Smith 3701* (SRP)	AF013694
C. mira Morley	Panama. *Smith 2450* (WIS)	AF013695
C. oblongifolia Rusby	Peru. Cuzco: Prov. Urubamba, Machu Picchu, 3 Apr. 1989, *Smith 1721* (WIS)	AF013696

Table 1. Continued.

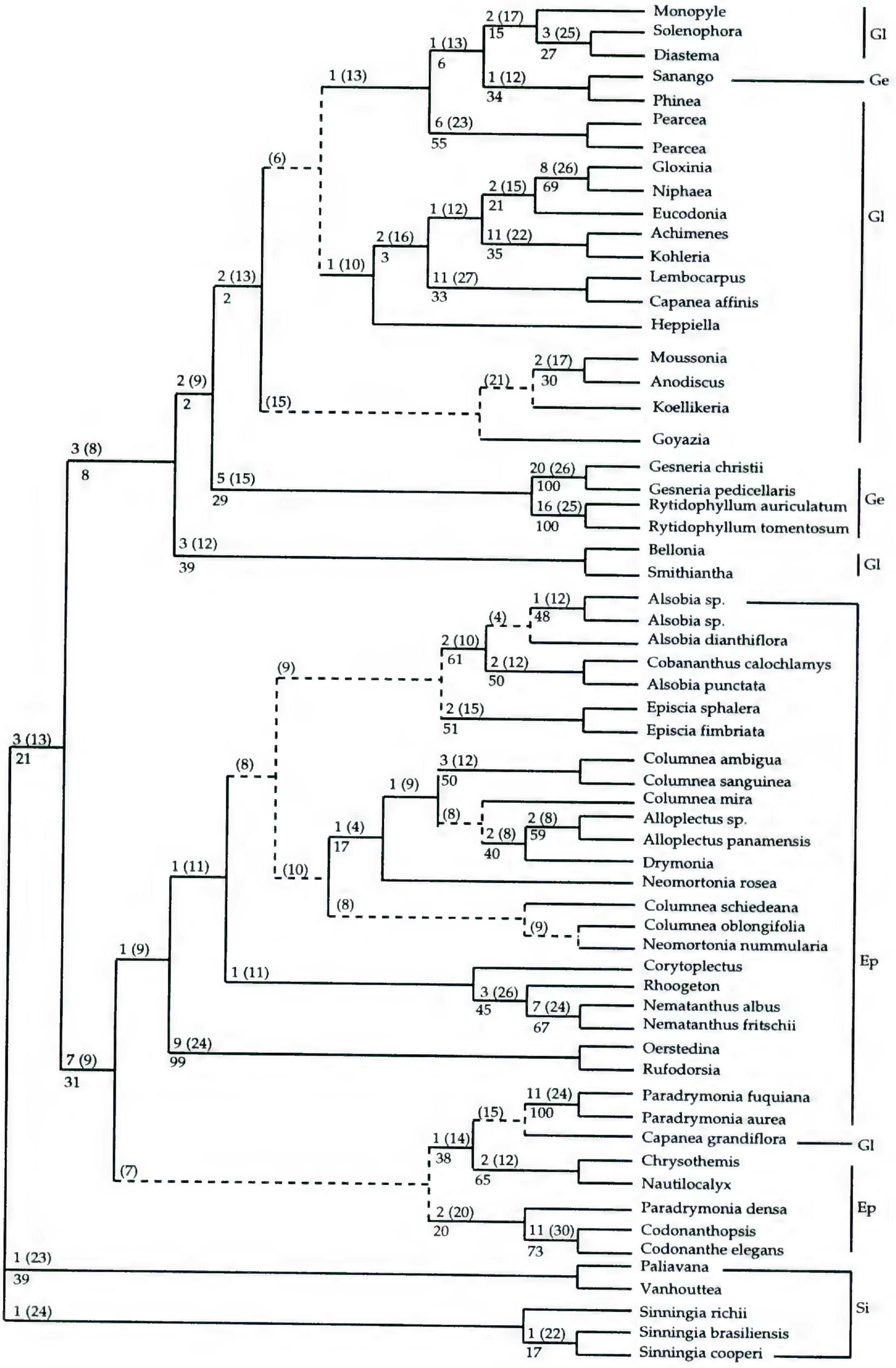
Species	Collection and voucher	GenBank number
C. sanguinea (Pers.) Hanst.	Cultivated. *Smith 3369* (SRP)	AF013697
C. schiedeana Schlecht.	Mexico. Vera Cruz: road from Xalapa to Missantla, *Smith 288* (WIS)	U62164
Corytoplectus speciosus (Poepp.) Wiehler	Ecuador. Near Limón, 15 May 1994 no voucher	AF013698
D. urceolata Wiehler	Ecuador. Napo: San Rafael Falls, 4 Apr. 1996, *Smith 3416* (SRP)	AF013699
Episcia fimbriata Fritsch	Cultivated. *Smith 3947* (SRP)	AF013700
E. sphalera Leeuw.	French Guiana. Mte. des Nouragues, 16 June 1994, *Feuillet et al. 94–079* (US)	AF013701
Nautilocalyx adenosiphon (Leeuw.) Wiehler	Cultivated. *Skog 7847* (US)	AF013702
Nematanthus albus Chautems, ined.	Brazil. 17 Jan. 1998, *Smith et al.* 3726 (SRP)	AF206197
N. fritschii Hoehne	Brazil. São Paulo: Mpo. Espiritu, 17 Jan. 1998, *Smith et al. 3720* (SRP)	AF206198
Neomortonia nummularia (Hanst.) Wiehler	Cultivated. *Smith 3944* (SRP)	AF013703
N. rosea Wiehler	Cultivated (US)	AF013704
Oerstedina cerricola Wiehler	Panama. Bocas del Toro: Cerro Pate Macho, 20 Nov. 1978, *Hammel 5754* (US)	AF206199
Paradrymonia aurea Wiehler	Cultivated. *Skog 7979* (US)	AF013705
P. densa (C. H. Wright) Wiehler	Cultivated. *Stewart s.n.* (SRP)	AF013706
P. fuquaiana Wiehler	Ecuador. 6 Jan. 1990, *Skog 7889* (US)	AF013707
Rhoogeton viviparus Lwbg.	Guyana. Potaro-Siparuni region, Kaieteur Falls, 18 Oct. 1987, *Kvist et al. 370* (US)	AF206200
Rufodorsia major Wiehler	Cultivated. *Skog 7761* (US)	AF013708
Outgroups: Sinningieae		
Paliavana prasinata (Ker-Gawl.) Fritsch	Brazil. *Skog 5399* (US)	U62174
Sinningia brasiliensis (Regel & Schmidt) Wiehler	Brazil. *Dunn 9104014* (SRP)	U62175
Sinningia cooperi (Paxt.) Wiehler	Cultivated. *Skog 7808* (US)	U62201
Sinningia richii Clayb.	Cultivated (US)	U62186
Vanhouttea lanata Fritsch	Cultivated. *Skog 7690* (US)	U62203

the placement of *Lembocarpus* in Gloxinieae. Reports of hybrids were apparently premature; no hybrids between these genera have been documented (Boggan, 1991).

Beaufort-Murphy (1983) placed *Lembocarpus* in Gloxinieae sensu Fritsch (1894) based on seed characteristics, noting that the seed shape was similar to some species of *Episcia* Mart. (tribe Episcieae) but not like any species of *Gloxinia*. Thus seed shape implies an affinity to Episcieae. Beaufort-Murphy (1983) also noted that the shape of the cells of the seed coat was similar to *Smithiantha* Kuntze, a member of Gloxinieae. Beaufort-Murphy (1983) concluded that *Lembocarpus* may have an isolated position within Gesnerioideae due to its anomalous combination of seed characteristics.

In summary, morphological data support the placement of *Lembocarpus* either in Gloxinieae or Episcieae. The similarity to Gloxinieae is based on the annular nectary, campanulate corolla, and shape of the cells of the seed coat. Its placement in Episcieae is largely based on the many characters it shares with *Rhoogeton*: superior ovary, tuber, calyx venation, inflorescence structure, acaulescent habit, and ovules only on outer placental surfaces (Leeuwenberg, 1958). The superior ovary and tuberous habit are unknown in Gloxinieae, but are common in Episcieae. In contrast, the annular nectary is known outside of Gloxinieae and Gesnerieae only in Beslerieae, a tribe with several other defining characters that would exclude the placement of *Lembocarpus*. Tubers are more widespread among members of Sinningieae (sensu Smith et al., 1997b) than elsewhere in Gesnerioideae, implying that *Lembocarpus* may best be placed among these genera. Tubers also are known from members of Episcieae (Wiehler, 1983) but not other Gloxinieae (sensu Smith et al., 1997b).

The tribe Gloxinieae has received recent attention with regard to the phylogenetic relationships of its genera (Smith & Atkinson, 1998). However, neither *Goyazia* nor *Lembocarpus* was included in


Monopyle
Solenophora
Diastema
Sanango
Phinea
Pearcea
Pearcea
Gloxinia
Niphaea
Eucodonia
Achimenes
Kohleria
Lembocarpus
Capanea affinis
Heppiella
Moussonia
Anodiscus
Koellikeria
Goyazia
Gesneria christii
Gesneria pedicellaris
Rytidophyllum auriculatum
Rytidophyllum tomentosum
Bellonia
Smithiantha
Alsobia sp.
Alsobia sp.
Alsobia dianthiflora
Cobananthus calochlamys
Alsobia punctata
Episcia sphalera
Episcia fimbriata
Columnea ambigua
Columnea sanguinea
Columnea mira
Alloplectus sp.
Alloplectus panamensis
Drymonia
Neomortonia rosea
Columnea schiedeana
Columnea oblongifolia
Neomortonia nummularia
Corytoplectus
Rhoogeton
Nematanthus albus
Nematanthus fritschii
Oerstedina
Rufodorsia
Paradrymonia fuquiana
Paradrymonia aurea
Capanea grandiflora
Chrysothemis
Nautilocalyx
Paradrymonia densa
Codonanthopsis
Codonanthe elegans
Paliavana
Vanhouttea
Sinningia richii
Sinningia brasiliensis
Sinningia cooperi
Gl
Ge
Gl
Ge
Gl
Ep
Gl
Ep
Si

previous cladistic analyses of molecular data due to the lack of leaf material. This study presents results regarding the phylogenetic position of these genera within the neotropical Gesneriaceae.

Materials and Methods

Voucher information and Genbank numbers for all sequences used in this analysis are presented in Table 1. DNA for *Goyazia* was isolated from silica gel dried material (Smith et al., 1992), and the *ndh*F gene was amplified in two overlapping sections (positions 1–1350, and 972–2044). DNA for *Lembocarpus* was obtained from herbarium specimens (Savolainen et al., 1995) using the DNEasy Plant miniprep kits (Qiagen) following the manufacturer's instructions. The *ndh*F gene for *Lembocarpus* was amplified in two overlapping fragments using primers 172–1350R and 972–2044R. The first fragment is smaller than those reported previously (Smith et al., 1997b) since amplifying the DNA from herbarium specimens required successive amplifications using internal primers. Initial amplifications followed DNA procedures described elsewhere (Smith et al., 1997b) using primers 1 and 1350R for the first part and 803 and 2044R for the second part. Subsequent amplifications required the use of 172 and 972 as forward primers, although the same reverse primers resulted in successful amplifications.

The focus of this analysis was on Gloxinieae since both *Goyazia* and *Lembocarpus* are currently classified in this tribe (Burtt & Wiehler, 1995). However, since there is considerable question regarding the tribal position of *Lembocarpus*, initial analyses were conducted using representatives of all tribes of the neotropical subfamily Gesnerioideae. Members of the Old World tribe Epithematae were used as the outgroup because some preliminary results implied a potential relationship to this tribe. Results from this preliminary analysis (not included) allowed for a more restricted taxon sampling that would permit greater analytical flexibility as well as minimizing homoplasy in the data set. Subsequent analyses used only Gloxinieae, Gesnerieae, and Episcieae as well as Sinningieae as outgroup. The choice of taxa for the reduced analysis considered all possible tribes where *Lembocarpus* may have affinities (Gloxinieae, Episcieae, and Sinningieae). Taxonomic choice derived from previous tribal analyses with Episcieae sister to Gloxinieae/Gesnerieae but Sinningieae sister to these three tribes (Smith et al., 1997b). The data matrix for all taxa contains 1.54% missing cells based on total sequence alignments.

Phylogenetic Analysis

Phylogenetic divergence was reconstructed using PAUP version 4.0d64 to implement maximum parsimony (MP) (Farris, 1970; Farris et al., 1970; Swofford & Maddison, 1987) and maximum likelihood (MLE). In this study, trees were generated using the general heuristic option. To search for islands of equally parsimonious trees (Maddison, 1991), the search strategy of Olmstead and Palmer (1994) was implemented searching for 1000 trees each in five subsequent analyses with the nearest neighbor interchange (NNI) search option in effect and mulpars "off." Each of the results from the five NNI searches was used as the starting tree(s) for a search with tree bisection reconnection (TBR) and mulpars "on." This search strategy was used for all MP analyses.

Because of the greater time involved in MLE analysis, a smaller sampling was utilized. In this analysis, a full sampling of Gloxinieae was used and one species each of *Gesneria* L. and *Rytidophyllum* Mart. (Gesnerieae). Representatives of Episcieae were used as the outgroup with taxa selected to include possible relatives of *Lembocarpus* (most notably *Rhoogeton*). MLE trees were generated using the heuristic search option with TBR and mulpars "on." Under the MLE option, the Hasegawa et al. (1985) model was used, which allows for unequal nucleotide frequencies and differential rates for transitions and transversions. The assumed nucleotide frequencies were estimated from the data: A = 0.27809, C = 0.15598, G = 0.17529, and T = 0.39063. MLE trees were compared to MP trees using the Kishino-Hasegawa test (Kishino & Hasegawa, 1989). Additional MP trees were generated using the same search criteria described above with the same taxa utilized in the MLE analysis.

Branch support analysis was performed to ex-

←

Figure 1. One of 48 maximum parsimony trees of 3998 steps each, CI = 0.38, RI = 0.33. These trees were rooted using Sinningieae. Numbers above clades are decay values; numbers in parentheses are branch lengths using the acctran option of PAUP. Numbers below clades indicate bootstrap values. Terminal branch lengths are not shown. Branches shown with dashed lines indicate nodes that are not supported in a strict consensus of all 48 trees. Abbreviations for tribes are: Ep = Episcieae, Ge = Gesnerieae, Gl = Gloxinieae, Si = Sinningieae.

amine trees that were one or two steps longer than the most-parsimonious trees (Bremer, 1988, 1994; Donoghue et al., 1992). Clades that persisted in strict consensus trees two steps beyond the most-parsimonious trees were examined using the constraints option to search for the shortest tree that did not contain that clade. Bootstrap analysis (Felsenstein, 1985) was performed using 100 replicates with TBR and mulpars "off" and "on." Because there is substantial morphological and biogeographic data that imply a relationship between *Lembocarpus* and *Rhoogeton*, the constraints option was used to assess the most-parsimonious tree with these genera as a monophyletic group. The trees generated from the constraints analysis were compared to the unconstrained trees using the Kishino-Hasegawa test (Kishino & Hasegawa, 1989).

Results

The initial analysis utilized all members of the Gesnerioideae with the Old World tribe Epithematae as the outgroup (not shown). The MP analysis resulted in 14 trees of 5128 steps each (trees not shown). In all of these trees both *Lembocarpus* and *Goyazia* were within Gloxinieae. MP analysis of the reduced data set resulted in 48 trees of 3998 steps each from three different islands of most-parsimonious trees, with consistency index (CI) = 0.38, and retention index (RI) = 0.33. One of these trees is presented in Figure 1 with dashed lines to indicate clades that collapse in the strict consensus of all trees. This tree is more resolved than prior results from *ndh*F analyses (Smith & Atkinson, 1998). These trees place both *Goyazia* and *Lembocarpus* within Gloxinieae although the position of *Goyazia* is not resolved; *Lembocarpus* is sister to *Capanea* Decne. ex Planch. As demonstrated in simulation studies (DeBry & Olmstead, 2000), the bootstrap values with mulpars "off" or "on" differed only by a few points (data not shown). The values on Figure 1 are from the analysis with mulpars "off."

An additional 14 steps beyond the most-parsimonious trees were necessary to force *Lembocarpus* and *Rhoogeton* into a monophyletic group (not shown). When forced together, *Lembocarpus* and *Rhoogeton* together are sister to *Nematanthus* Schrader (Episcieae). The Kishino-Hasegawa test (Kishino & Hasegawa, 1989) did not indicate a significant difference between the constrained and unconstrained trees.

MLE analysis resulted in a -ln likelihood of 16118.64716 (Fig. 2). *Goyazia* and *Lembocarpus* are both in Gloxinieae, and *Lembocarpus* is sister to *Capanea* as indicated by parsimony. Further, *Goyazia* is sister to a clade containing *Moussonia* Regel, *Anodiscus* Benth., and *Koellikeria* Regel (Fig. 2). An MP analysis of the same taxa used in the MLE analysis resulted in two islands for a total of four trees of 2076 steps each (trees not shown). The Kishino-Hasegawa test (Kishino & Hasegawa, 1989) did not indicate a significant difference between the MP and MLE trees.

Discussion

Goyazia

The results of this study support the placement of *Goyazia* in Gloxinieae (Figs. 1, 2). Wiehler (1983) was the first to place *Goyazia* in his Gloxinieae, where it has remained in a modern revised version of the classification system (Burtt & Wiehler, 1995). Although *Goyazia* is always in a clade with *Moussonia*, *Anodiscus*, and *Koellikeria*, its exact relationship to these other genera is unresolved (Fig. 1). One of the trees from the MP analysis (Fig. 1) and MLE analysis indicate that *Goyazia* is sister to these three genera (Fig. 2), and *Koellikeria* is sister to *Moussonia* and *Anodiscus*. However, *Goyazia* and *Koellikeria* are both found in Brazil, whereas *Moussonia* is endemic to Central America, and *Anodiscus* is native to the Andes of Peru. Since the majority of Gloxinieae are found west of the Andes, the most-parsimonious explanation for this distribution would be two separate migrations to Brazil, one each for *Goyazia* and *Koellikeria*, if the relationships among these genera are accurate as seen in Figure 2. However, weak bootstrap and decay index support herein for these relationships implies that more data are necessary before conclusions regarding the biogeography of these taxa can be made.

Lembocarpus

As with *Goyazia*, the results of this analysis confirm the placement of *Lembocarpus* in Gloxinieae (Figs. 1, 2). This placement is more controversial since early descriptions of *Lembocarpus* noted its disparate calyx venation, inflorescence, acaulescent habit, outer ovule placentation, superior ovary, and tubers (Leeuwenberg, 1958) as shared with *Rhoogeton* (Episcieae).

Placement of *Rhoogeton* in Episcieae is in itself controversial. Although morphological data and *ndh*F sequences (Smith, 2000b) provide evidence for its placement there, ITS sequences imply its position outside of Episcieae and possibly within Gloxinieae (Smith, 2000b). Unfortunately, ITS se-

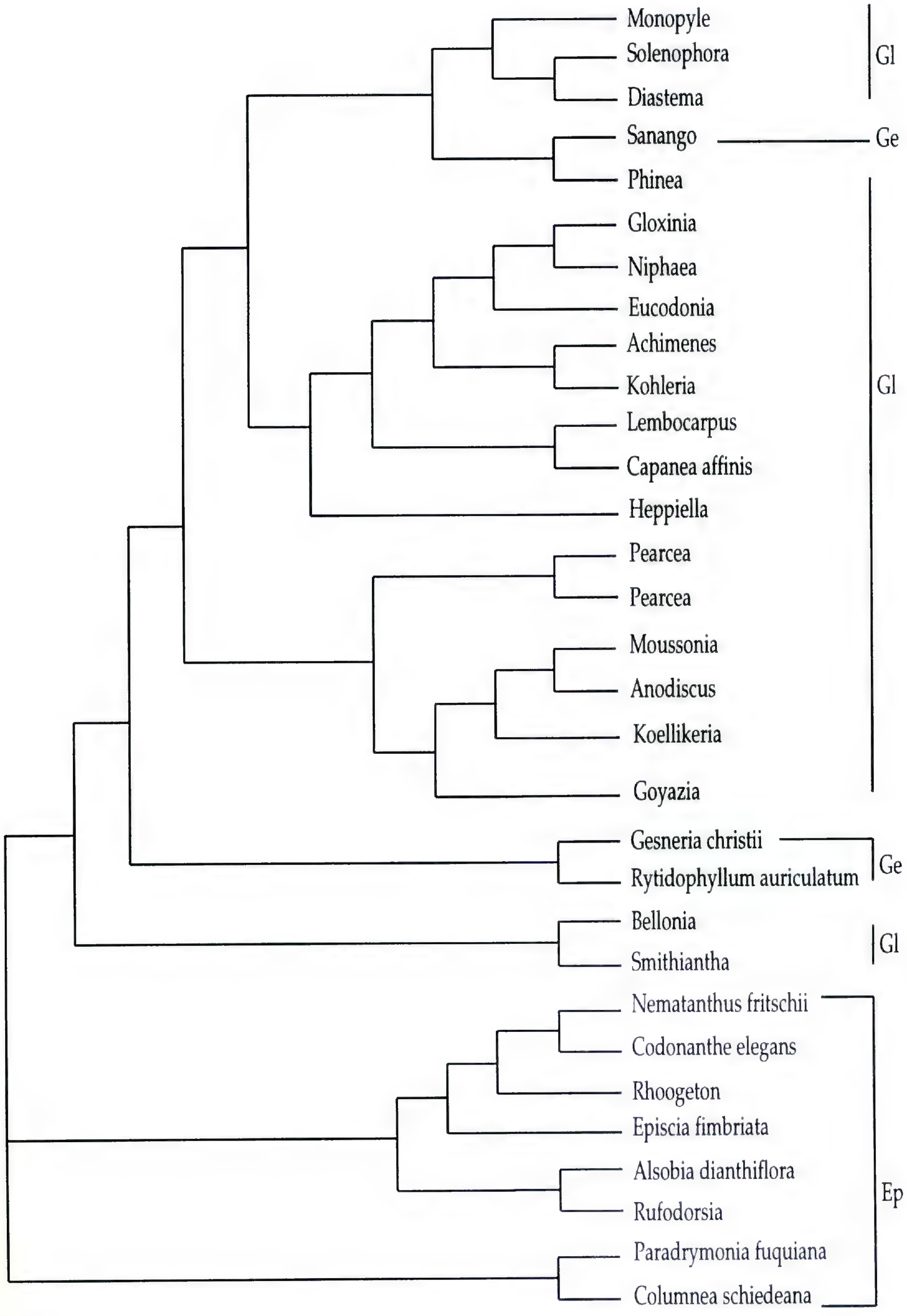


Figure 2. Maximum likelihood estimate tree, -ln likelihood = 16118.64716. To minimize computer time this analysis used only representative taxa of Episcieae as outgroups. Abbreviations for tribes are: Ep = Episcieae, Ge = Gesnerieae, Gl = Gloxinieae.

quences could not be obtained using the DNA from herbarium material used for *Lembocarpus* in this study. Despite the several morphological character states shared between *Lembocarpus* and *Rhoogeton*, 14 additional steps beyond the most-parsimonious trees are necessary to place *Lembocarpus* and *Rhoogeton* in a monophyletic group using *ndh*F sequence data. Thus, regardless of the tribal affinity of *Rhoogeton*, it does not appear to be closely related to *Lembocarpus* based on *ndh*F data.

Morphological support for the placement of *Lembocarpus* in Gloxinieae comes from the corolla and seed. Although Beaufort-Murphy (1983) hypothesized that *Lembocarpus* may have an isolated position within the Gesnerioideae due to a unique combination of characters, this combination of seed surface characters and superior ovary is best viewed as autapomorphic. The shape of the cells of the seed coat and campanulate corolla may be synapomorphies to place *Lembocarpus* in Gloxinieae.

An additional synapomorphy for *Lembocarpus* and Gloxinieae is nectary shape. Whereas the annular nectary is widespread in Gloxinieae, it also is known from tribes Gesnerieae and Beslerieae. In cladistic analyses of Gloxinieae and Gesnerieae based on *ndh*F sequences, the position of Gesnerieae always creates a paraphyletic Gloxinieae (Figs. 1, 2; Smith & Atkinson, 1998). This implies that if not derived from within Gloxinieae, the Gesnerieae are closely related, and the annular nectary is likely a synapomorphy for both tribes Gloxinieae and Gesnerieae. The Beslerieae are more distantly related to Gloxinieae based on previous phylogenetic analyses of morphology and *ndh*F sequences. Beslerieae are best considered one of the more earlier lineages within Gesnerioideae (Smith, 1996; Smith et al., 1997b; Smith, 2000a). Therefore the annular nectary of Beslerieae is unlikely homologous to the annular nectary of Gloxinieae/Gesnerieae. Although nectary structure may be a homoplastic character, it still serves as a synapomorphy to unite Gloxinieae and Gesnerieae, as well as to place *Lembocarpus* in this group.

CAPANEA AND *LEMBOCARPUS*

The data presented here not only provide new evidence for the tribal placement of *Lembocarpus*, but place it as sister to *Capanea* (Figs. 1, 2). This is one of the more strongly supported clades in the tree based on decay index values. This relationship of *Lembocarpus* to *Capanea* is unusual in that *Capanea* is the only epiphytic genus of Gloxinieae, whereas *Lembocarpus* is a terrestrial, tuberous, acaulescent herb. No striking synapomorphies unite these genera with the exception that they are among the few genera within Gloxinieae that lack scaly rhizomes. The placement of *Capanea* in Gloxinieae contradicts earlier cladistic analyses of *ndh*F sequences of Gloxinieae where it was placed in Episcieae (Smith & Atkinson, 1998). The earlier analysis based on *ndh*F sequences only used *Capanea grandiflora* (Kunth) Decne. ex Planch., and *Capanea affinis* is added to the present analysis. The source of discrepancy is as yet unknown, but based on results of ITS and *ndh*F sequences (Smith, 2000b), it is clear that *Capanea affinis* belongs in Gloxinieae.

Summary

The *ndh*F data presented here confirm the placement of both *Goyazia* and *Lembocarpus* within Gloxinieae. *Lembocarpus* is strongly supported by decay index, moderate (relatively) bootstrap values, and MLE analysis as sister to *Capanea*. *Goyazia* is placed in a clade with *Moussonia*, *Anodiscus*, and *Koellikeria* in both MP and MLE analyses, although the relationships among these four genera are not fully resolved in MP trees. Because *ndh*F seems to be at the limits of its resolving power within Gloxinieae (also observed in tribe Episcieae; Smith & Carroll, 1997; Smith, 2000b), it will be essential in future investigations to add additional sequence data to resolve more fully the intergeneric relationships of this tribe.

Literature Cited

Beaufort-Murphy, H. T. 1983. The seed surface morphology of the Gesneriaceae utilizing the scanning electron microscope and a new system for diagnosing seed morphology. Selbyana 6: 220–422.

Boggan, J. K. 1991. A morphological study and cladistic analysis of *Sinningia* and associated genera with particular reference to *Lembocarpus*, *Lietzia*, *Paliavana* and *Vanhouttea* (Gesneriaceae: Gloxinieae). M.S. Thesis, Cornell University, Ithaca, New York.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

———. 1994. Branch support and tree stability. Cladistics 10: 295–304.

Burtt, B. L. & H. Wiehler. 1995. Classification of the family Gesneriaceae. Gesneriana 1: 1–4.

DeBry, R. W. & R. G. Olmstead. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. Syst. Biol. 49: 171–179.

Donoghue, M. J., R. G. Olmstead, J. F. Smith & J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann. Missouri Bot. Gard. 79: 333–345.

Farris, S. J. 1970. Methods for computing Wagner trees. Syst. Zool. 19: 83–92.

———, A. G. Kluge & M. J. Eckardt. 1970. A numerical

approach to phylogenetic systematics. Syst. Zool. 19: 172–191.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Fritsch, K. 1893–1894. Gesneriaceae, *In*: A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien, Vol. 4(3b): 133–185. Wilhelm Engelmann, Leipzig.

Hasegawa, M., H. Kishino & T. Yano. 1985. Dating of the human-ape splitting by a molecular clock. J. Molec. Evol. 22: 412–419.

Kishino, H. & M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Molec. Evol. 31: 170–179.

Kvist, L. P. 1990. Revision of *Heppiella* (Gesneriaceae). Syst. Bot. 15: 720–735.

——— & L. E. Skog. 1988. The genus *Cremosperma* (Gesneriaceae) in Ecuador. Nordic J. Bot. 8: 259–269.

——— & ———. 1989. Revision of *Reldia* (Gesneriaceae). Nordic J. Bot. 8: 601–611.

——— & ———. 1993. The genus *Columnea* (Gesneriaceae) in Ecuador. Allertonia 6: 327–400.

——— & ———. 1996. Revision of *Pearcea* (Gesneriaceae). Smithsonian Contr. Bot. 84: 1–47.

Leeuwenberg, A. J. M. 1958. The Gesneriaceae of Guiana. Acta Bot. Neerl. 7: 291–444.

Maddison, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst. Zool. 40: 315–328.

Olmstead, R. G. & J. D. Palmer. 1994. Chloroplast DNA systematics: A review of methods and data analysis. Amer. J. Bot. 81: 1205–1224.

Savolainen, V., P. Cuénod, R. Spichiger, M. D. P. Martinez, M. Crèvecoeur & J. F. Manen. 1995. The use of herbarium specimens in DNA phylogenetics: Evaluation and improvement. Pl. Syst. Evol. 197: 87–98.

Smith, J. F. 1994. Systematics of *Columnea* section *Pentadenia* and section *Stygnanthe* (Gesneriaceae). Syst. Bot. Monogr. 44: 1–89.

———. 1996. Tribal relationships within the Gesneriaceae: A cladistic analysis of morphological data. Syst. Bot. 21: 497–513.

———. 2000a. A phylogenetic analysis of tribes Beslerieae and Napeantheae (Gesneriaceae): Parsimony and maximum-likelihood analyses of *ndh*F sequences. Syst. Bot. 25: 71–80.

———. 2000b. Phylogenetic resolution within the tribe Episcieae (Gesneriaceae): Congruence of ITS and *ndh*F sequences from parsimony and maximum-likelihood analyses. Amer. J. Bot. 87: 883–897.

———. 2000c. An assessment of three data sets in phylogenetic analysis: Tribal relationships within the Gesneriaceae as a model. Pl. Syst. Evol. 221: 179–198.

——— & S. Atkinson. 1998. Phylogenetic analysis of the tribes Gloxinieae and Gesnerieae (Gesneriaceae): Data from *ndh*F sequences. Selbyana 19: 122–131.

——— & C. L. Carroll. 1997. Phylogenetic relationships of the Episcieae (Gesneriaceae) based on *ndh*F sequences. Syst. Bot. 22: 713–724.

——— & K. J. Sytsma. 1994a. Evolution in the Andean epiphytic genus *Columnea* (Gesneriaceae), Part I. Morphological variation. Syst. Bot. 19: 220–235.

——— & ———. 1994b. Evolution in the Andean epiphytic genus *Columnea* (Gesneriaceae), Part II. Chloroplast DNA restriction site variation. Syst. Bot. 19: 317–336.

——— & ———. 1994c. Molecules and morphology: Congruence of data in *Columnea* (Gesneriaceae). Pl. Syst. Evol. 194: 37–52.

———, ———, J. S. Shoemaker & R. L. Smith. 1992. A qualitative comparison of total cellular DNA extraction protocols. Phytochem. Bull. Bot. Soc. Amer. 23: 2–9.

———, K. D. Brown, C. L. Carroll & D. S. Denton. 1997a. Familial placement of *Cyrtandromoea*, *Titanotrichum*, and *Sanango*: Three problematic genera of the Lamiales. Taxon 46: 65–74.

———, J. C. Wolfram, K. D. Brown, C. L. Carroll & D. S. Denton. 1997b. Tribal relationships in the Gesneriaceae: Evidence from DNA sequences of the chloroplast gene *ndh*F. Ann. Missouri Bot. Gard. 84: 50–66.

Soltis, D. E., P. S. Soltis, T. G. Collier & M. L. Edgerton. 1991. Chloroplast DNA variation within and among genera of the *Heuchera* group: Evidence for chloroplast capture and paraphyly. Amer. J. Bot. 78: 1091–1112.

Swofford, D. L. & W. P. Maddison. 1987. Reconstructing ancestral character states under Wagner parsimony. Math. Biosci. 87: 199–229.

Wiehler, H. 1983. A synopsis of the neotropical Gesneriaceae. Selbyana 6: 1–249.

MORFOLOGÍA DEL POLEN DE LAS ESPECIES ARGENTINAS DE *BAUHINIA* (CERCIDEAE, CAESALPINIOIDEAE, FABACEAE)[1]

Juan C. Gamerro[2] *y Renée H. Fortunato*[3]

Resumen

Las especies del género *Bauhinia* L. son variables en el hábito y en la morfología de la flor, del polen y del fruto, lo que llevó a diferentes autores a adoptar distintos criterios en su delimitación taxonómica. Con el empleo del microscopio fotónico, incluyendo contraste de interferencia y el electrónico de barrido se ha estudiado los granos de polen de las 7 especies que crecen en la Argentina. Este análisis ha permitido agruparlos en tipos y subtipos morfológicos distintos: *Tipo 1:* 3-zonocolporados, tectados, tectum microgranuloso con procesos supratectales esféricos rugosos = *B. microstachya* (Raddi) Benth. var. *microstachya*. *Tipo 2*: 3-zonocolporados a porados, microrreticulados, semitectum con procesos supratectales espiniformes = *B. uruguayensis* Benth. *Tipo 3*: 4–6-zonocolpados, reticulados, semitectum con procesos supratectales gemados; *Subtipo a*: Colpos largos y angostos = *B. argentinensis* Burkart var. *argentinensis*, *B. argentinensis* Burkart var. *megasiphon* (Burkart) Fortunato y *B. bauhinioides* (Mart.) Macbr.; *Subtipo b*: Colpos relativamente cortos y anchos = *B. mollis* (Bong.) D. Dietr. var. *notophila* (Griseb.) Fortunato, *B. forficata* Link subsp. *pruinosa* (Vogel) Fortunato & Wunderlin y *B. affinis* Vogel. Al mismo tiempo se comprueba la existencia de una correlación aceptable entre los caracteres del polen y las categorías infragenéricas establecidas por Wunderlin et al. en 1987.

Abstract

The species of *Bauhinia* L. are variable in their habit, flower, pollen, and fruit morphology. This led several authors to adopt different criteria for the taxonomic delimitation of the genus. With the aid of brightfield, interference contrast, and scanning electron microscopy, the pollen of the 7 Argentinean species has been studied. This analysis has allowed the pollen to be grouped into different types and subtypes: *Type 1*: 3-zonocolporate, tectate, tectum microgranulate with supratectal spherical rugulate processes = *B. microstachya* (Raddi) Benth. var. *microstachya*. *Type 2*: 3-zonocolporate to porate, microreticulate, semitectum with spinose supratectal processes = *B. uruguayensis* Benth. *Type 3:* 4–6-zonocolpate, reticulate, semitectum with geminate supratectal processes; *Subtype a*: Colpi long and narrow = *B. argentinensis* Burkart var. *argentinensis*, *B. argentinensis* Burkart var. *megasiphon* (Burkart) Fortunato, and *B. bauhinioides* (Mart.) Macbr.; *Subtype b*: Colpi wide and short = *B. mollis* (Bong.) D. Dietr. var. *notophila* (Griseb.) Fortunato, *B. forficata* Link subsp. *pruinosa* (Vogel) Fortunato & Wunderlin, and *B. affinis* Vogel. At the same time, a correlation was confirmed between pollen morphology and the infrageneric divisions established by Wunderlin et al. in 1987.

Key words: Argentina, *Bauhinia*, Caesalpinioideae, Cercideae, Fabaceae, pollen morphology, taxonomy.

El género *Bauhinia* L. está representado por aproximadamente 300 especies de distribución pantropical y subpantropical; de ellas cerca de 150 crecen en las regiones neotropicales y subneotropicales. Las especies de este género poseen gran variación en el hábito y en la morfología de la flor y del fruto, lo que llevó a diferentes autores a adoptar distintos criterios en la delimitación taxonómica a nivel genérico e infragenérico. A pesar de ello, en la mayoría de los trabajos más recientes se acepta al género *Bauhinia* en sentido amplio, siendo la clasificación más moderna la propuesta por Wunderlin et al. (1987).

La morfología del polen presenta una notoria va-

[1] Se agradece a Nélida M. Bacigalupo por la lectura crítica del manuscrito y a Alejandra Gardini por las copias de las ilustraciones realizadas en scanner. Asimismo, este agradecimiento se hace extensivo al Instituto de Neurobiología, CONICET, a la Facultad de Ciencias Exactas, Químicas y Naturales, Universidad de Morón y al Instituto de Botánica Darwinion por las observaciones y fotografiadas realizadas al MEB, y a los curadores de los herbarios citados por el préstamo del material estudiado.

[2] Instituto de Botánica Darwinion, Casilla de Correo 22, 1642 San Isidro, Buenos Aires, Argentina.

[3] Investigadora del CONICET en el Instituto de Recursos Biológicos, INTA, 1712 Castelar, Buenos Aires, Argentina.

riación en la forma y tamaño de los granos, en la ornamentación de la exina y en el tipo y número de aperturas. Estos caracteres fueron utilizados por varios autores para relacionarlos con las clasificaciones taxonómicas establecidas (Melhem & Salgado-Labouriau, 1963; Smith, 1964; Palacios Chavez, 1970; Schmitz, 1973; Larsen, 1975; Larsen & Larsen, 1983; Ferguson & Pearse, 1986; Ferguson, 1990). Si bien los estudios señalan cierto paralelismo entre los caracteres del polen y de la macromorfología, se han citado casos de formas polínicas similares en especies poco relacionadas y formas con diferencias marcadas entre otros taxones afines (Ferguson, 1990).

Bauhinia en Argentina posee una distribución geográfica marginal, con siete especies y una variedad (Fortunato, 1984, 1986, 1996; Fortunato & Wunderlin, 1985), las que representan tres de los cuatro subgéneros propuestos en la clasificación de Wunderlin et al. (1987) (Cuadro 1).

La morfología polínica de algunos de los taxa aquí considerados fue analizada parcialmente por varios autores (Schmitz, 1973; Barth & Bouzada, 1964; Ferguson & Pearse, 1986; Ferguson, 1990). En esta contribución se presenta un detallado estudio del polen de las especies que crecen en Argentina y se lo relaciona con las divisiones infragenéricas propuestas por Wunderlin et al. (1987).

Materiales y Métodos

Las muestras de polen fueron obtenidas de ejemplares de distintos herbarios, los cuales son citados para cada especie siguiendo las abreviaturas adoptadas en Holmgren et al. (1990). Cuando no se dispuso de suficiente material de origen argentino se hizo uso de ejemplares coleccionados en países limítrofes.

La mayoría del material fue acetolizado según el método de Erdtman (1960); posteriormente, una porción de los granos acetolizados fue separada y sometida al proceso de clorinación (Erdtman, 1960). En el caso de *B. argentinensis*, *B. bauhinioides*, *B. forficata* subsp. *pruinosa*, *B. mollis* var. *notophila* y *B. affinis* por presentar los granos poca resistencia a la acetólisis y sufrir fuertes deformaciones fue necesario estudiarlas con el método de Wodehouse (1935).

En la observación al microscopio electrónico de barrido (MEB) para eliminar impurezas superficiales, las muestras de las 5 especies del subgénero *Bauhinia* fueron tratadas con la mezcla acetolítica en frío y lavadas con agua destilada; posteriormente, se sometieron al secado por punto crítico y se montaron sobre portaespecímenes con una capa delgada de adhesivo. En las restantes especies: *B. uruguayensis* y *B. microstachya* los granos fueron acetolizados, lavados con agua destilada y suspendidos en alcohol etílico 70°; una o dos gotas de la suspensión se dejaron evaporar sobre un trozo de película fotográfica no expuesta y fijada para eliminar las sales de plata. Los trozos de película con los granos fueron pegados con pintura conductiva a los portaespecímenes. En ambos casos los materiales fueron metalizados con oro-paladio. Las observaciones se realizaron en los microscopios: JEOL JSM 35CF del Instituto de Neurobiología, CONICET, Leitz AMR 1200 de la Facultad de Ciencias Exactas, Químicas y Naturales, Universidad de Morón y Zeiss DSM 940 A del Instituto de Botánica Darwinion, CONICET.

Los cortes fueron realizados incluyendo los granos sin acetolizar en goma arábiga-glicerina según el método de Leins modificado por Hideux y Marceau (1972) y cortados con micrótomo de deslizamiento Reichert. Para las observaciones al microscopio fotónico (MF) se efectuaron cortes de 1–2 μm de espesor, se tiñeron con fucsina básica y se montaron en Euparal; ocasionalmente, cortes de algunas especies fueron analizados en microscopio de contraste de interferencia (CI); los estudiados al microscopio electrónico de barrido (MEB) se realizaron con un espesor de aproximadamente 10 μm.

La terminología utilizada es la desarrollada por Erdtman (1972) y Punt et al. (1994).

El estudio de la estratificación de la exina de *B. affinis* se efectuó sobre granos acetolizados/clorinados montados en glicerina-hidrato de cloral 1:4. Para expresar la densidad de distribución de los procesos supratectales de las especies del subgénero *Bauhinia*: *B. argentinensis*, *B. affinis*, *B. forficata* subsp. *pruinosa*, *B. mollis* var. *notophila* y *B. bauhinioides*, se ha calculado un índice que expresa la relación entre el número de procesos proyectados en el amb en corte óptico ecuatorial y el diámetro ecuatorial del grano; cuanto mayor es el número de procesos supratectales en el grano más alto será dicho índice. La medición de los granos fue efectuada sobre material acetolizado en *B. microstachya* y *B. uruguayensis* y en las demás especies montado según Wodehouse (1935). En el tamaño de los granos y de los procesos supratectales se citan medidas mínimas, medias y máximas, siendo la media el promedio de 25 granos por muestra estudiada.

Los preparados de las muestras de polen están depositados en la colección palinológica del Instituto de Botánica Darwinion.

Cuadro 1. Caracteres diferenciales polínicos y macromorfológicos entre los grupos taxonómicos.

Subg. *Phanera* sect. *Schnella*		*B. microstachya* var. *microstachya*	*Tipo 1* 3-zonocolporados tectum microgranuloso procesos supratectales esféricos			Lianas o arbustos escandentes con zarcillos	• Racimos compuestos o simples, terminales y axilares. • Cáliz campanulado. • Androceo con filamentos, libres. • Gineceo brevemente estipitado.	• Flores perfectas, pequeñas. • Ovario 1--2(3)-ovulado. • Estilo corto.	• Pétalos ovados a elípticos. • Estambres fértiles 10 sin apéndice ligular en la región basal.	Legumbre membranácea o papirácea, indehiscente, 1--2-seminada
Subg. *Elayuna* sect. *Benthamia*		*B. uruguayensis*	*Tipo 2* 3-zonocolporados a porados microrreticulados semitectum procesos supratectales espiniformes			Arboles o arbustos erguidos con espinas		• Flores perfectas–estaminadas, grandes. • Ovario 5--8-ovulado. • Estilo desarrollado en las flores perfectas.	• Pétalos obovados. • Estambres fértiles 10 sin apéndice ligular en la región basal.	Legumbre leñosa, tardíamente dehiscente, 5--8(9)-seminada
Subg. *Bauhinia* sect. *Pauletia*	Ser. *Cansenia*	*B. mollis* var. *notophila* *B. forficata* subsp. *pruinosa* *B. affinis*	*Tipo 3* (4-)5—6-zonocolpados reticulados procesos supratectales gemados	*Subtipo b* Colpos cortos y anchos	Relación N° de procesos supratectales del amb en vista polar/diámetro ecuatorial: < 0.17	Arboles o arbustos erguidos o semi apoyantes con acúleos infraestipulares	• Racimos paucifloros, extraxilares. • Cáliz espatáceo. • Androceo con filamentos connatos en la base. • Gineceo con ginopodio desarrollado.	• Flores perfectas, grandes. • Ovario pluriovulado. • Estilo siempre desarrollado.	• Pétalos anchos. • Estambres 10 fértiles con apéndice ligular en la región basal.	Legumbre leñosa, dehiscente, pluriseminada
	Ser. *Pentandra*	*B. argentinensis* var. *argentinensis* *B. argentinensis* var. *megasiphon*		*Subtipo a* Colpos largos y angostos	Relación N° de procesos supratectales del amb en vista polar/diámetro ecuatorial: > 0.17				• Pétalos lineares. • Estambres (4) 6--10 fértiles sin apéndice ligular en la región basal.	
	Ser. *Perlebia*	*B. bauhinioides*								Legumbre coriácea, indehiscente con disepimentos interseminales, pluriseminada

Resultados

Bauhinia microstachya var. *microstachya* (fig. 1a–g)

Granos de polen tricolporados, raramente sincolporados, suboblatos a oblato-esferoidales; diámetros: P = 29 (37) 47 μm, E = 35 (44) 53 μm; relación P/E: 0.77–0.92. Amb circular a ligeramente subtriangular (Fig. 1A). Colpos de límites difusos, casi tan largos como el diámetro polar, a veces fusionados en el polo, formados por anchas depresiones con ornamentación similar a los mesocolpios, pero menos compacta (Fig. 1B). Endoaperturas de 4 × 3.5 μm a 8 × 6 μm de diámetro, subcirculares con un delgado anillo externo (Fig. 1C).

Exina de 2.3–2.7 μm de espesor, tectada (Fig. 1D–G). Ectexina más o menos igual a la endexina, adelgazada hacia las endoaperturas (Fig. 1E–G). Tectum de ± 0.6 μm de espesor, microgranulado, microperforado? con procesos supratectales esféricos de 2 (3.5) 6 μm de diámetro y superficie rugulada (Fig. 1B, D); capa basal de menor espesor que el tectum, dejando entre ambas un estrecho infratectum con finas columelas, escasamente visibles al MF (Fig. 1F, G). Endexina engrosada hacia las endoaperturas de superficie interna microfoveolada (Fig. 1D, E, G).

Observación. Con excepción de Barth y Bouzada (1964) que dan tamaños ligeramente mayores, las características del polen de *B. microstachya* var. *microstachya* descriptas en este estudio, coinciden con lo citado por Schmitz (1973) y Ferguson y Pearce (1986). En una especie afín de Brasil: *B. smilacina* (Schott) Steud., se observó la presencia de un tectum densamente microperforado (Ferguson & Pearce, 1986: 291, fig. 47; Ferguson, 1990: 80, figs. 42, 43). Este carácter no se ha podido constatar fehacientemente en *B. microstachya* var. *microstachya* por no haberse realizado estudios con microscopio electrónico de transmisión (MET), no obstante el corte observado al MEB (Fig. 1D, E) sugeriría su existencia.

Material estudiado. BRASIL. **Rio de Janeiro:** sin loc., 1879, *Glaziou 10691* (LE); sin loc., s.f., *Martius Herb. Fl. Bras. 132* (LE). PARAGUAY. **Alto Paraná:** "in regione fluminis", 1909/10, *Fiebrig 6201* (LIL). ARGENTINA. **Misiones:** Depto. San Pedro, Santa Rosa, Tobuna, 15 Feb. 1952, *Capurro 1011* (BA).

Bauhinia uruguayensis (fig. 2a–g)

Granos de polen tricolporados a porados, oblato-esferoidales a esferoidales; diámetro: P = 41 (54) 61 μm, E = 47 (56) 64 μm; relación P/E: 0.88–1.02. Amb circular. Colpos generalmente cortos, mal definidos, con límites difusos, a veces desarrollándose de un solo lado de la endoapertura (Fig. 2C), membrana colpar granulada. Endoaperturas circulares de 7–10 μm de diámetro, con bordes ligeramente salientes (Fig. 2A, C).

Exina (Fig. 2B) de 2.7–3 μm de espesor, semitectada, microrreticulada con procesos supratectales espiniformes de 1.2 (2.4) 5.8 μm long. × 1.2 (2) 3.5 μm de diámetro (Fig. 2A, C, D–F); lúmenes de forma y tamaño irregular (Fig. 2B). Ectexina más o menos igual a la endexina, no se diferencia capa basal (Fig. 2D–F). Semitectum e infratectum formados por columelas dispuestas densamente que originan un microrretículo superficial (Fig. 2B). Endexina de superficie interna rugulada, ligeramente adelgazada hacia la endoapertura (Fig. 2D, E, G).

Observación. Los colpos son poco visibles y difíciles de medir observándose en muchos granos de un mismo ejemplar colpos medianamente desarrollados o ausentes (Fig. 2A, C).

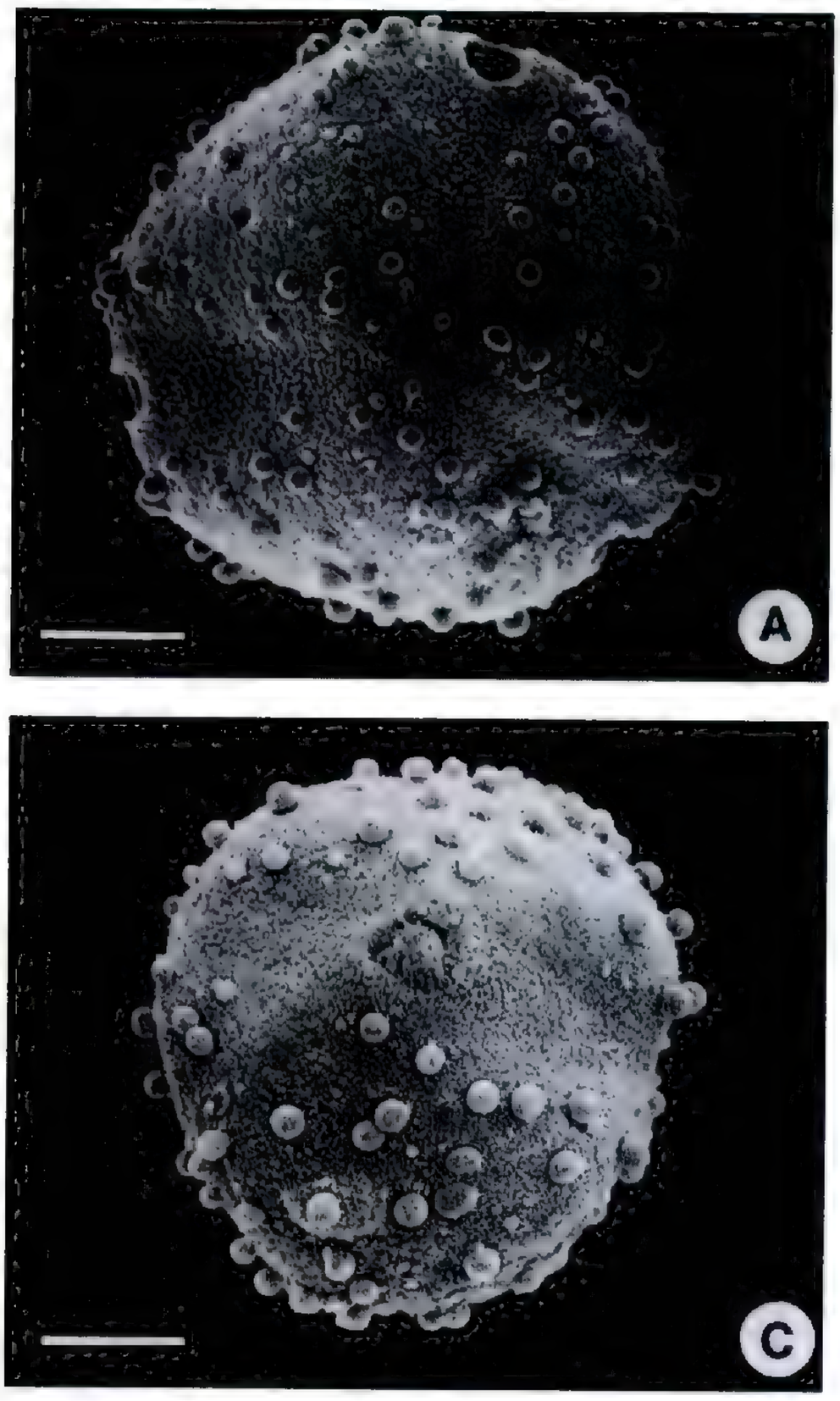
La morfología descripta de esta especie coincide con la ilustración y caracterización parcial realizada por Ferguson (1990). Este autor estudió un duplicado de uno de los ejemplares analizados en esta contribución (*Schinini 19942*).

Material estudiado. BRASIL. **Santa Catarina:** Dourado, Itapiranga, 3 Mar. 1964, *Klein 5232* (SI). ARGENTINA. **Misiones:** Depto. San Javier, Ayo. Pindaití, s.f., *Schwarz 4117* (LIL). **Corrientes:** Depto. Santo Tomé, Ayo. Chimiray y río Uruguay, 12 Nov. 1974, *Schinini 19942* (SI).

Bauhinia argentinensis var. *argentinensis* (fig. 3a–g)

Granos de polen (4) 5–6-zonocolpados, oblatos a oblato-esferoidales; diámetro: P = 82 (109) 125 μm, E = 108 (122) 134 μm; relación P/E: 0.70–1. Amb: tetra- penta- hexagonal a subcircular. Colpos largos y delgados de 71–88 μm long. × 6–15 μm lat., membrana cubierta por restos irregulares de ectexina (Fig. 3A–C).

Exina de 2.7–3 μm de espesor, semitectada, reticulada, heterobrocada con lúmenes irregulares (Fig. 3B, C), muros simplibaculados, con procesos supratectales gemados de 5 (5.5) 8 μm long. × 5 (5.4) 8 μm lat. (Fig. 3D). Ectexina de 2–2.3 μm de espesor. Semitectum generalmente más delgado que la capa basal; capa basal de 0.5–0.7 μm de espesor (Fig. 3F, G). Endexina aproximadamente igual a la capa basal, de ± 0.6 μm de espesor con superficie interna escabrosa (Fig. 3E–G).



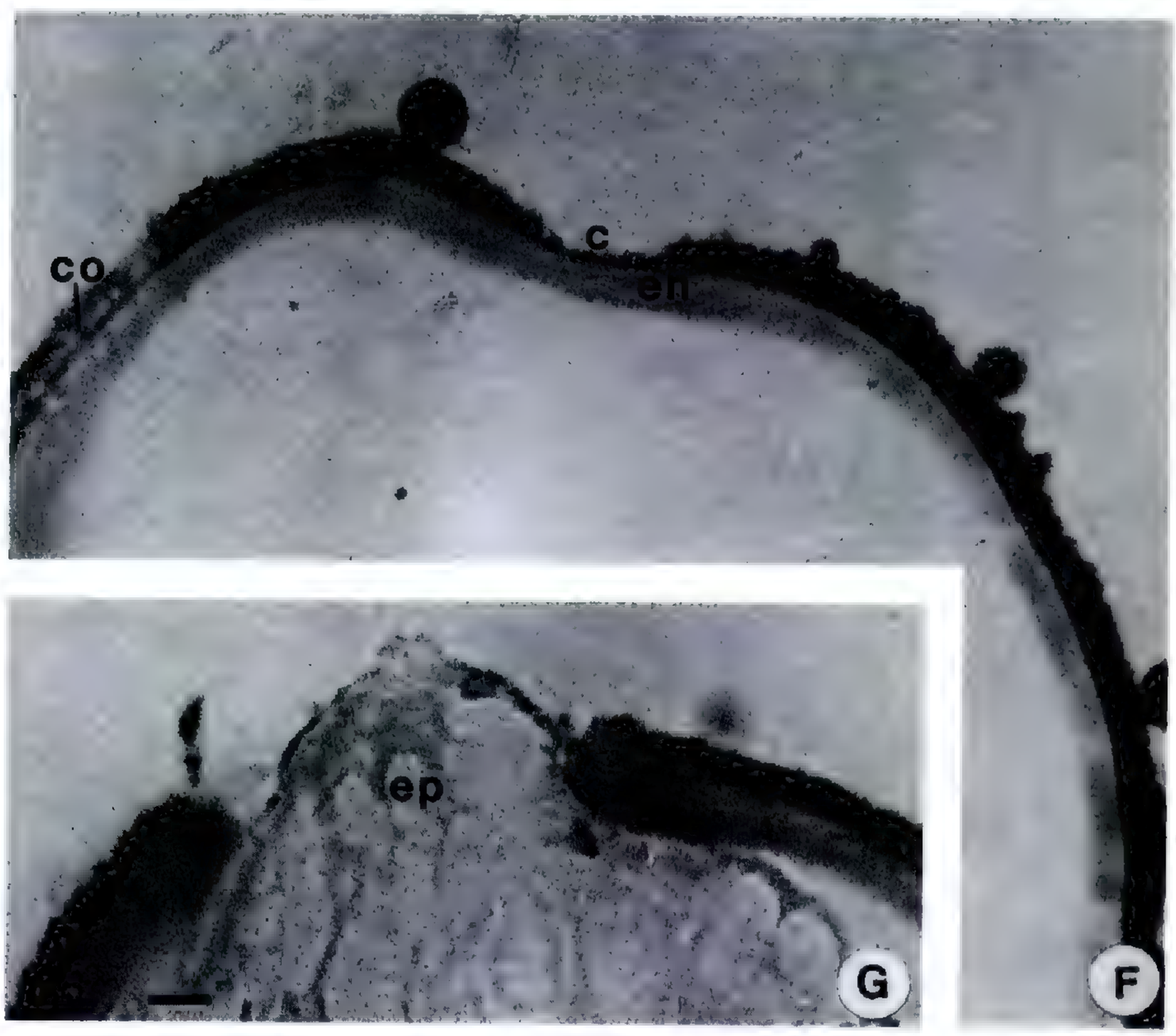


Figura 1. A–C, F, G, *Bauhinia microstachya* var. *microstachya* (*Capurro 1011*, BA); D, E (*Martius 132*, LE). —A. Grano en vista polar. —B. Detalle de apocolpio con los colpos casi fusionados en los polos. —C. Grano sin acetolizar en vista ecuatorial. —D. Corte de la exina. —E. Corte a través de la endoapertura: endexina engrosada en los bordes. —F y G. Estratificación de la exina en corte delgado, en G corte a través de la endoapertura: endexina engrosada en los bordes. c = colpo; co = columelas; ec = ectexina (tectum, infratectum y capa basal); ep = endoapertura; n = nexina gruesa con superficie interna microfoveolada; s = sexina. Fotomicrografías: A–E: MEB; F, G: MF. Escalas: A, C = 10 μm; B, D, E = 1 μm; F, G = 2 μm.

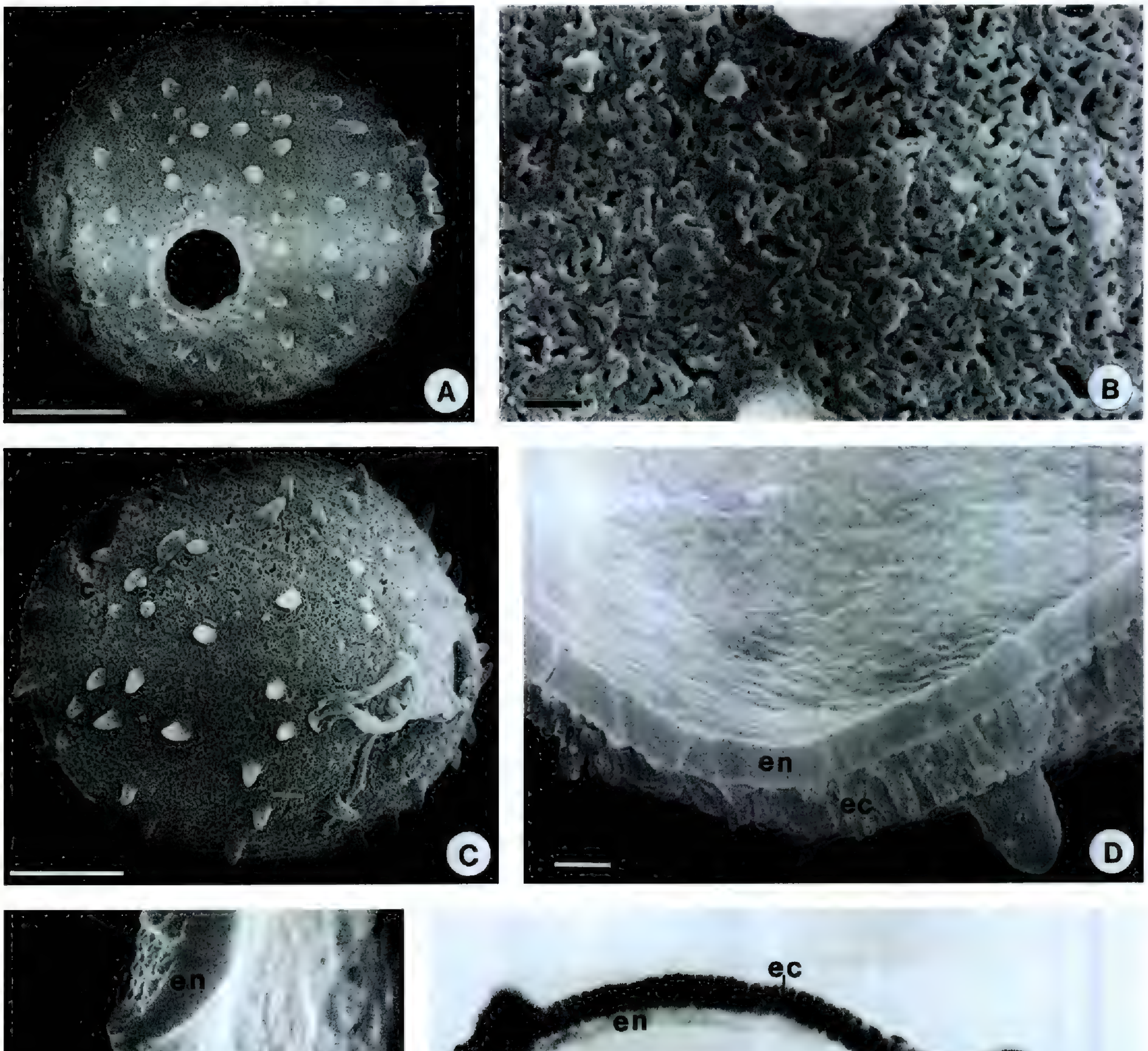


Figura 2. A–G, *Bauhinia uruguayensis* (*Schinini 19942*, CTES). —A. Grano en vista ecuatorial. —B. Detalle de la superficie microrreticulada. —C. Grano en vista ecuatorial oblícua. —D. Corte de la exina. —E. Corte a través de la endoapertura: bordes algo salientes presentando endexina ligeramente adelgazada. —F y G. Estratificación de la exina en corte delgado, en G a través de la endoapertura. c = colpo; ec = ectexina, en = endexina con superficie interna escabrosa. Fotomicrografías: A–E: MEB; F, G: MF. Escalas: A, C = 10 μm; B, D, E = 1 μm; F, G = 2 μm.

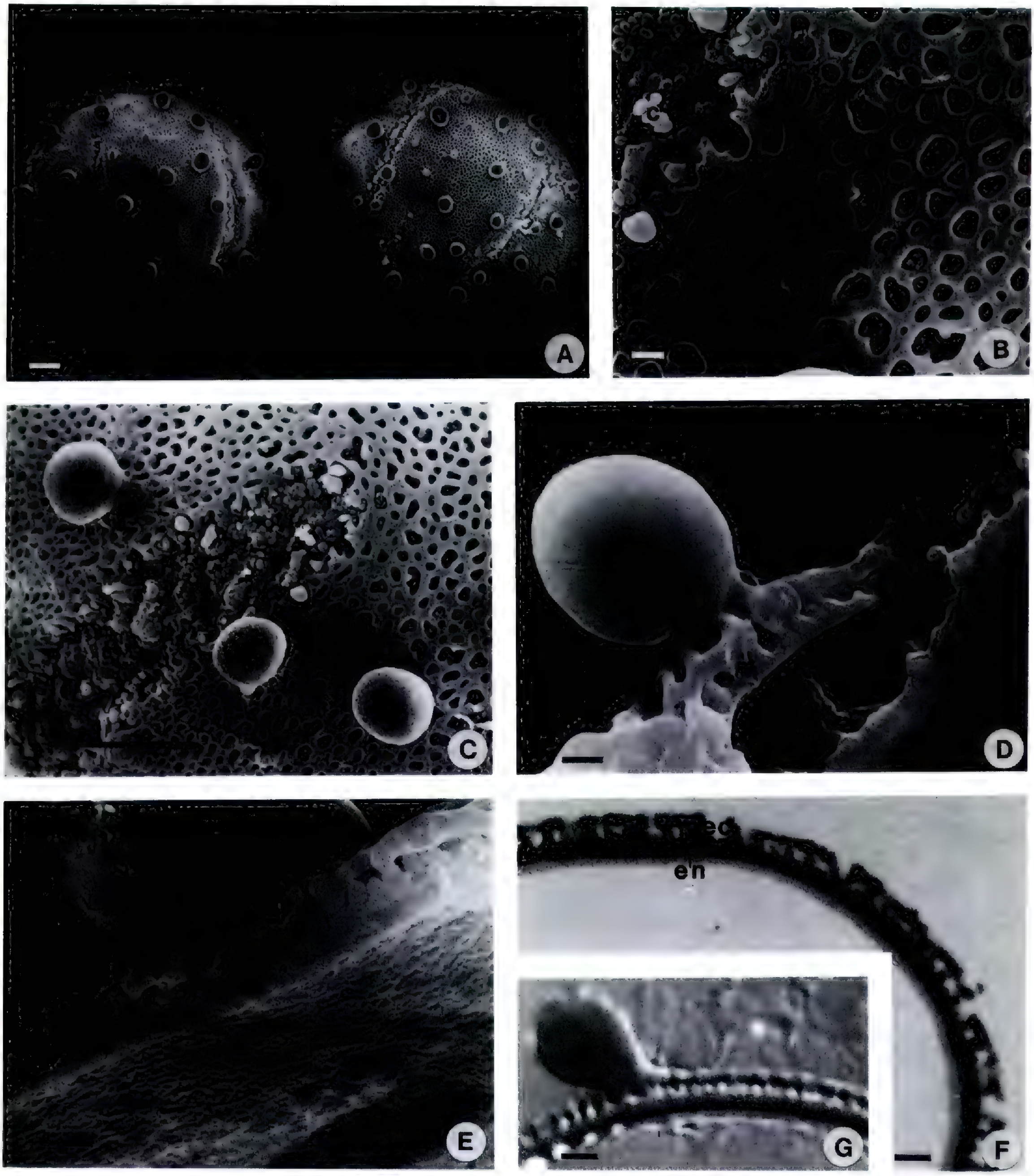


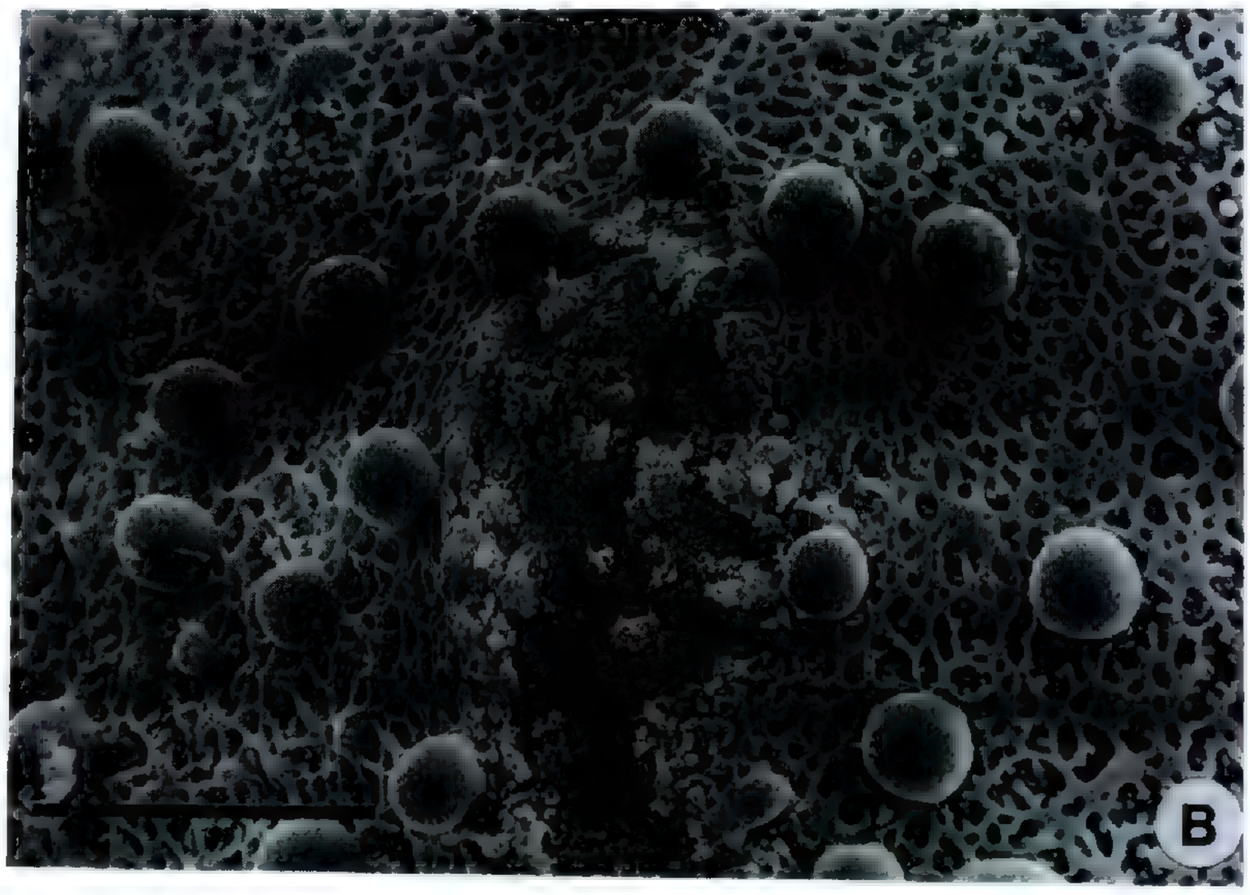
Figura 3. A–G, *Bauhinia argentinensis* var. *argentinensis* (*Rojas 2111*, SI). —A. Granos en vista ecuatorial. —B. Detalle de la ornamentación de la exina y parte de un colpo. —C. Detalle del extremo de un colpo. —D. Estratificación y ornamentación de exina fracturada. —E. Corte de la exina. —F y G. Estratificación de la exina en corte delgado. c = colpo; ec = ectexina; en = endexina con superficie interna escabrosa; n = nexina. Fotomicrografías: A–E: MEB; F: MF; G: CI. Escalas: A, C = 10 μm; B, D, E = 1 μm; F, G = 2 μm.

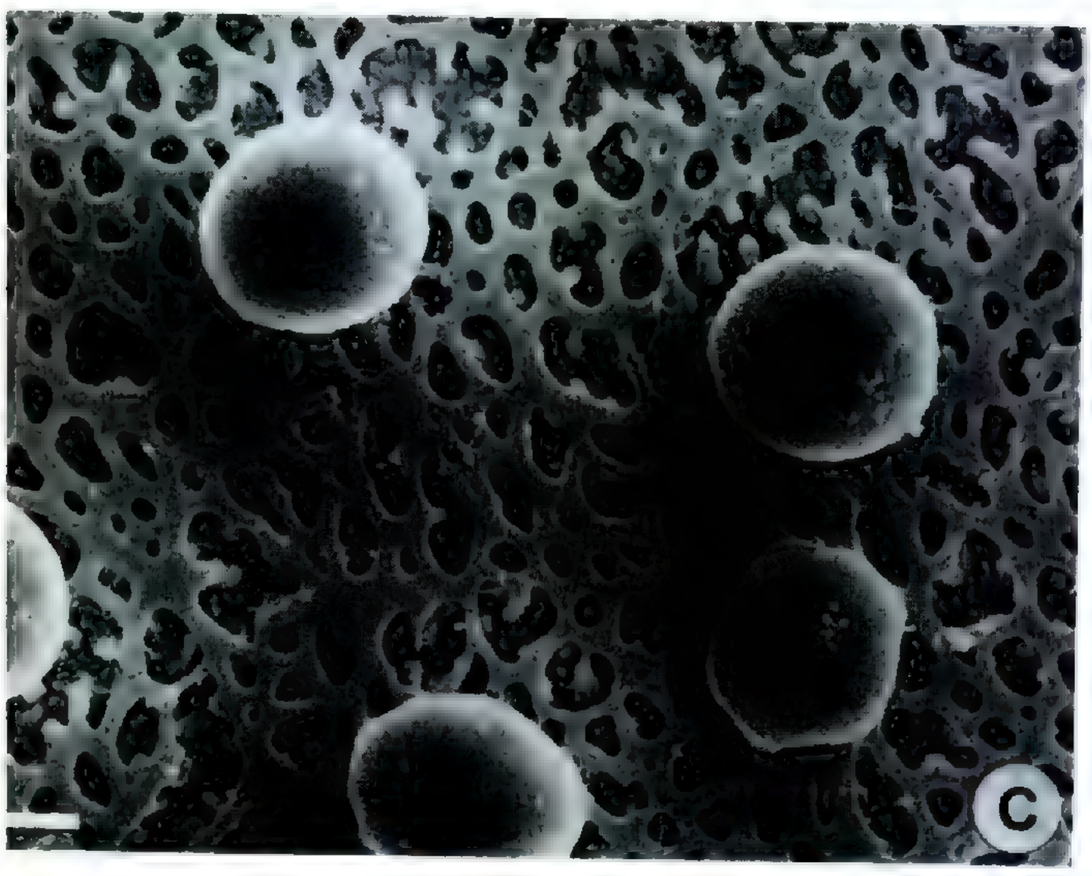
Relación Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial: 0.11 (0.18) 0.24.

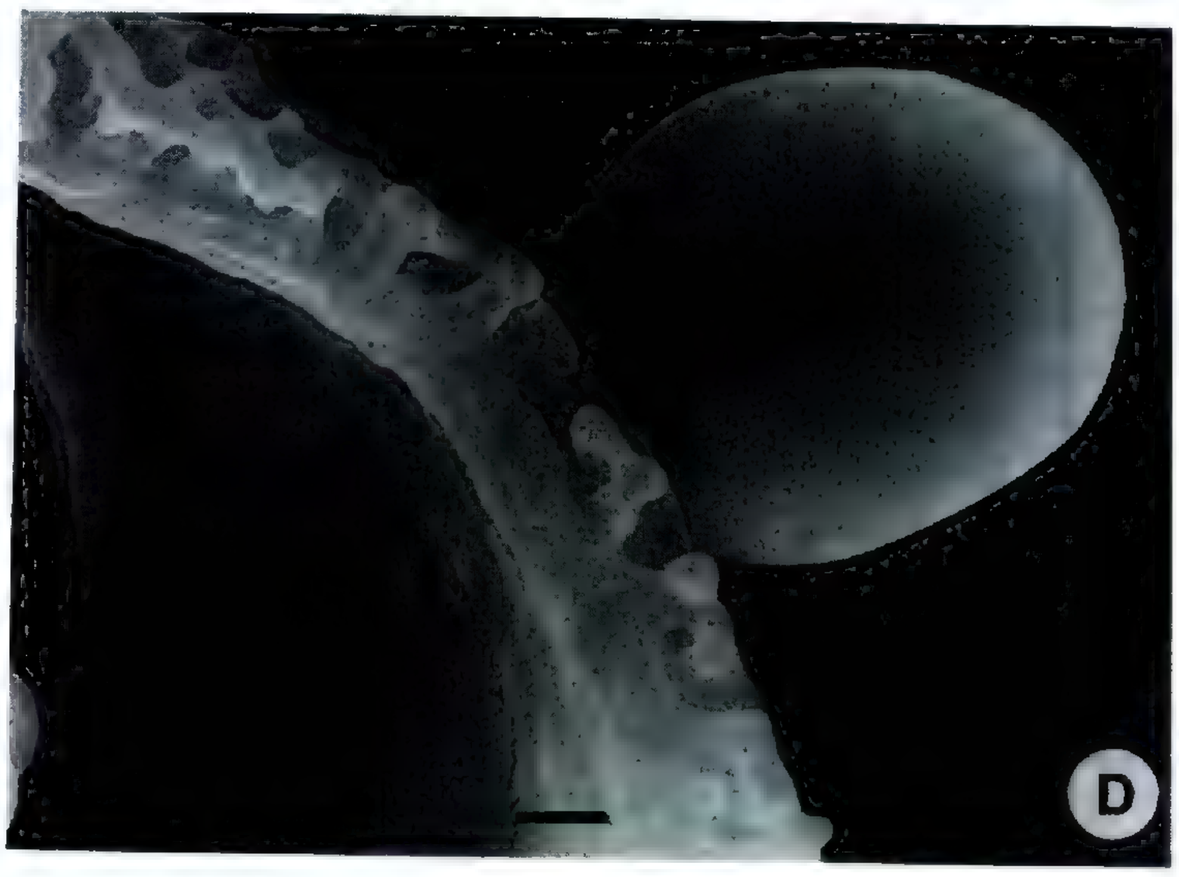
Material estudiado. PARAGUAY. **Chaco:** Puerto Casado, montes ribereños, Dec. 1916, *Rojas 2111* (SI); Chaco, cult. en Jardín Botánico a través de semillas procedentes del chaco, Feb. 1943, *Pavetti & Rojas 10414* (SI). **Boquerón:** Colonia Fernheim, colonia 22, 22°14′S, 60°12′W, Feb. 1981, *Arenas 1817* (SI).

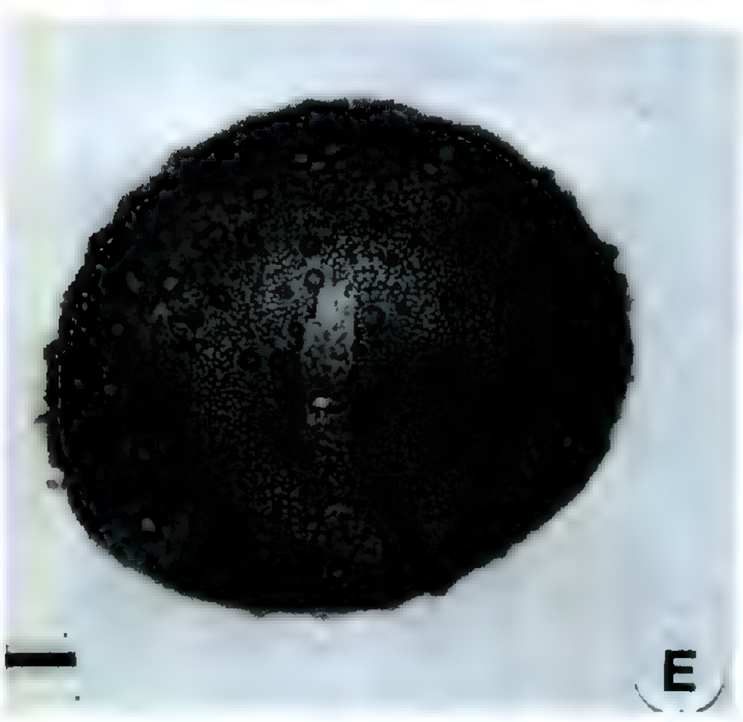
BAUHINIA ARGENTINENSIS VAR. *MEGASIPHON*

Los granos presentan la misma morfología que la descripta para la var. *argentinensis*; presentando el material observado diámetro: P = 82 (97) 109 μm, E = 106 (118) 126 μm; relación P/E: 0.75–0.91; colpos de 62–85 μm long. × 6–10 μm lat.; pro-









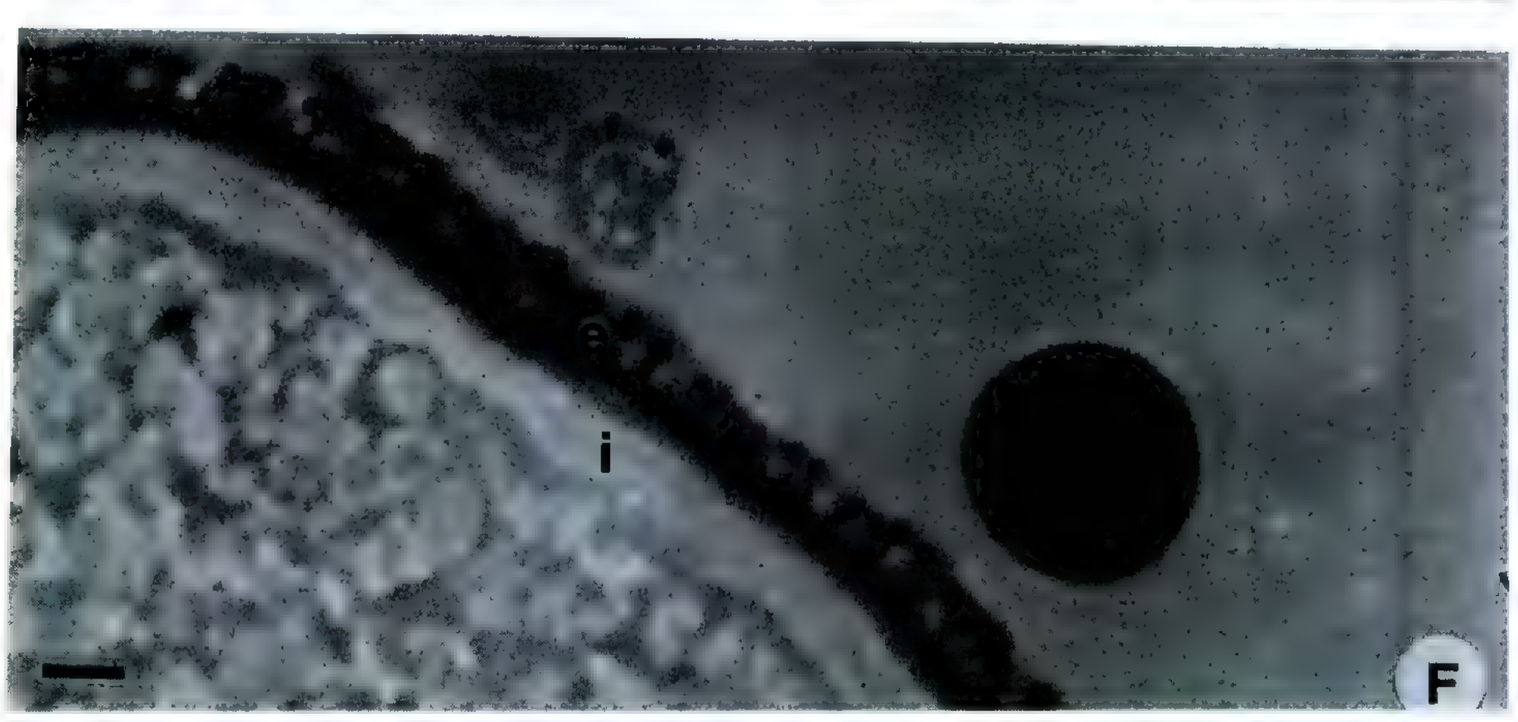


Figura 4. A–C, E, *Bauhinia bauhinioides* (*Jorgensen 2699*, SI); D, F (*Kermes 33*, BAF). —A. Grano en vista ecuatorial. —B. Detalle del extremo de un colpo. —C. Detalle de la ornamentación de la exina. —D. Corte de la exina: estratificación y ornamentación. —E. Grano en vista ecuatorial: colpo interrumpido por un puente de ectexina. —F. Estratificación de la exina en corte delgado; grano sin acetolizar. e = exina; i = intina. Fotomicrografías: A–D: MEB; E, F: MF (E montado según Wodehouse, 1935). Escalas: A, B = 10 μm; C, D = 1 μm; E = 20 μm; F = 2 μm.

cesos supratectales gemados de 3.4 (6) 10 μm long. × 2 (6) 9 μm lat.

Relación Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial: 0.14 (0.16) 0.23.

Material estudiado. BOLIVIA. **Chuquisaca:** Luis Calvo, El Salvador Cimbol, 3 Feb. 1992, *Saravia Toledo & Joaquín 10556* (CTES).

BAUHINIA BAUHINIOIDES (FIG. 4A–F)

Granos de polen (4–)5–6-zonocolpados, suboblatos a oblato-esferoidales; diámetro: P = 88 (103) 118 μm, E = 106 (118) 129 μm; relación P/E: 0.77–0.97. Amb: circular a ligeramente penta-, hexagonal. Colpos largos y delgados de ± 62–68 μm long. × 5–9 μm lat., oblongos, obtusos a re-

dondeados en los extremos, membrana granulada con restos irregulares de ectexina (Fig. 4A, B, E).

Exina de 2.3–2.7 μm de espesor, semitectada, reticulada, heterobrocada con lúmenes irregulares, muros simplibaculados, con procesos supratectales gemados de 0.6 (3.9) 7 μm long. × 1.20 (4) 8 μm lat. (Fig. 4B–D). Ectexina de 1.7–2 μm de espesor. Semitectum y capa basal menor o igual a la endexina (Fig. 4F). Endexina de 0.6–0.7 μm de espesor (Fig. 4D, F).

Relación Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial: 0.17 (0.25) 0.33.

Observación. La caracterización polínica coincide con la breve descripción realizada por Schmitz (1973, sub nom. *Pauletia bauhinioides* (Mart.) Schmitz) y la estratificación de la exina con el corte al MET, publicado por Ferguson y Pearce (1986: fig. 25).

En ocasiones los colpos están interrumpidos por cortos puentes de ectexina (Fig. 4E).

Material estudiado. BRASIL. **Mato Grosso do Sul:** Campo Grande, Corumbá, pantanal, s.f., *Mautone 633* (RB). ARGENTINA. **Formosa:** sin loc., 5 Nov. 1900, *Kermes 33* (SI); Yuquerí Negro, Dec. 1917, *Jorgensen 2699* (SI).

BAUHINIA MOLLIS VAR. *NOTOPHILA* (FIG. 5A–G)

Granos de polen (4?–) 5–6-zonocolpados, suboblatos; diámetro: P = 94 (110) 147 μm, E = 109 (131) 159 μm; relación P/E: 0.79–0.86. Amb subcircular a penta-hexagonal. Colpos relativamente cortos y anchos de 32–59 μm long. × 12–20 μm lat., subelípticos a suboblongos, membrana con pocos restos irregulares de ectexina (Fig. 5A, D, E).

Exina de 2.9–3.4 μm de espesor, semitectada, reticulada, heterobrocada con lúmenes irregulares, muros simplibaculados con procesos supratectales gemados de 3.5 (7.5) 13.8 μm long. × 3 (7) 11.5 μm lat. (Fig. 5B, D, G). Ectexina 2–2.5 μm de espesor. Endexina de 0.9 μm de espesor; capa basal igual o un poco mayor que la endexina; semitectum de espesor ligeramente menor que la capa basal (Fig. 5B, F, G).

Relación Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial: 0.14 (0.15) 0.17.

Material estudiado. ARGENTINA. **Jujuy:** Dpto. Ledesma, Calilegua, camino camino a los cafetales, 11 Feb. 1980, *Cabrera et al. 31492* (SI). **Salta:** Dpto. San Martín, de Coronel Cornejo hacia Altos de San Antonio, bordeando el gasoducto, 8 km de Cornejo, 12 Feb. 1986, *Zuloaga 2742* (SI); Dpto. Orán, Orán, Río Piedras, 26 Nov. 1911, *M. Rodriguez 116* (SI).

BAUHINIA FORFICATA SUBSP. *PRUINOSA* (FIG. 6A–G)

Granos de polen (4–)5–6-zonocolpados, oblatos a oblato-esferoidales; diámetro: P = 85 (108) 118 μm, E = 103 (120.5) 138 μm; relación P/E: 0.72–0.92. Amb subcircular a penta-hexagonal. Colpos cortos y anchos de 32–56 μm long. × 9–23 μm lat., subelípticos a suboblongos, membrana con restos irregulares de ectexina (Fig. 6A, B).

Exina de 3 a 3.4 μm de espesor, semitectada, reticulada, heterobrocada con lúmenes irregulares, muros simplibaculados, con procesos supratectales gemados de 4.6 (9) 13.8 μm long. × 3.5 (8) 13 μm lat. (Fig. 6C, D). Ectexina de 2.3–2.5 μm. Endexina de 0.7–0.9 μm de espesor con superficie interna escabrosa (Fig. 6E). Capa basal y semitectum más o menos igual a la endexina (Fig. 6F, G).

Relación Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial: 0.15–(0.15) 0.16.

Observación. La morfología polínica observada en esta contribución ratifica la breve descripción efectuada por Schmitz (1973 sub nom. *Pauletia candicans* (Benth.) A. Schmitz).

Según Barth y Bouzada (1964) el polen de *B. forficata* subsp. *forficata* (NE al SE de Brasil) es oblato-esferoidal y porado, sin embargo al estudiar material de esta subespecie (*Guedes 477* y *Reitz C2152*, SI), no se observó diferencias con la subsp. *pruinosa*.

Material estudiado. ARGENTINA. **Misiones:** Dpto. Eldorado, El Dorado, Salto Elena, 6 ene. 1972, *A. Fernandez et al. 40* (SI); Dpto. Guaraní, Fracrán, 15 Feb. 1978, *Cabrera & Saenz 29221* (SI). **Tucumán:** alrededores de la Capital, 31 ene. 1919, *Schreiter 409* (SI).

BAUHINIA AFFINIS (FIG. 7A, B, D–F)

Granos de polen 5–6-zonocolpados, oblato-esferoidal a oblatos; diámetro: P = 73 (88) 97 μm, E = 91 (105) 120 μm; relación P/E: 0.75–0.93. Amb pentagonal o hexagonal a subcircular. Colpos cortos y anchos de 23–38 μm long. × 9–21 μm lat., subelípticos a suboblongos, membrana con restos de ectexina (Fig. 7A, D, E).

Exina de 2.9–3.5 μm de espesor, semitectada, reticulada, homobrocada con lúmenes de forma irregular, muros simplibaculados, con procesos supratectales geminados de 2.3 (5.5) 8 μm long. × 2.3 (5) 8 μm lat. (Fig. 7B). Ectexina de ± 2.5 μm de espesor. Endexina de ± 0.9 μm de espesor (Fig. 7F).

Relación Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial: 0.08 (0.12) 0.14.

Observación. En esta especie no se efectuaron

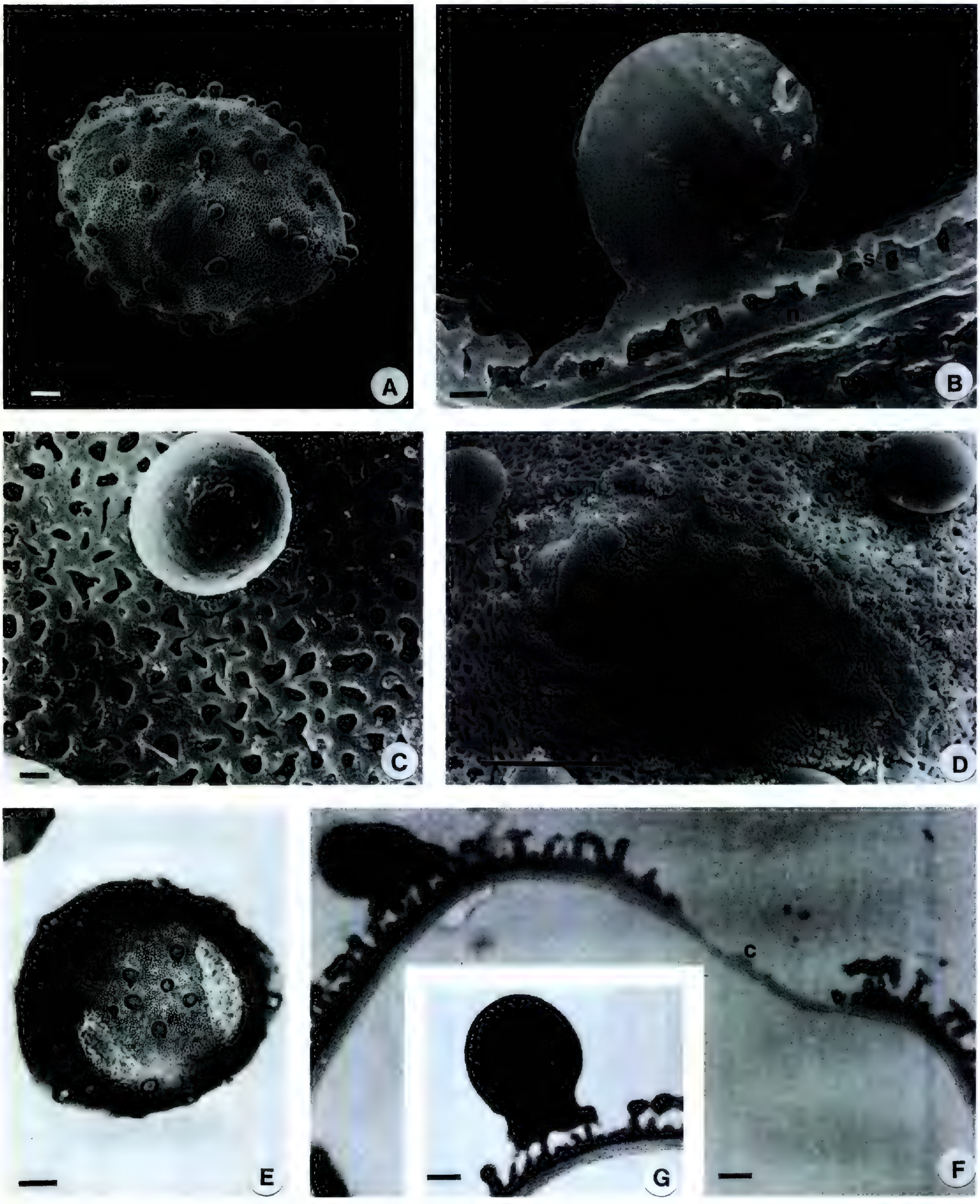
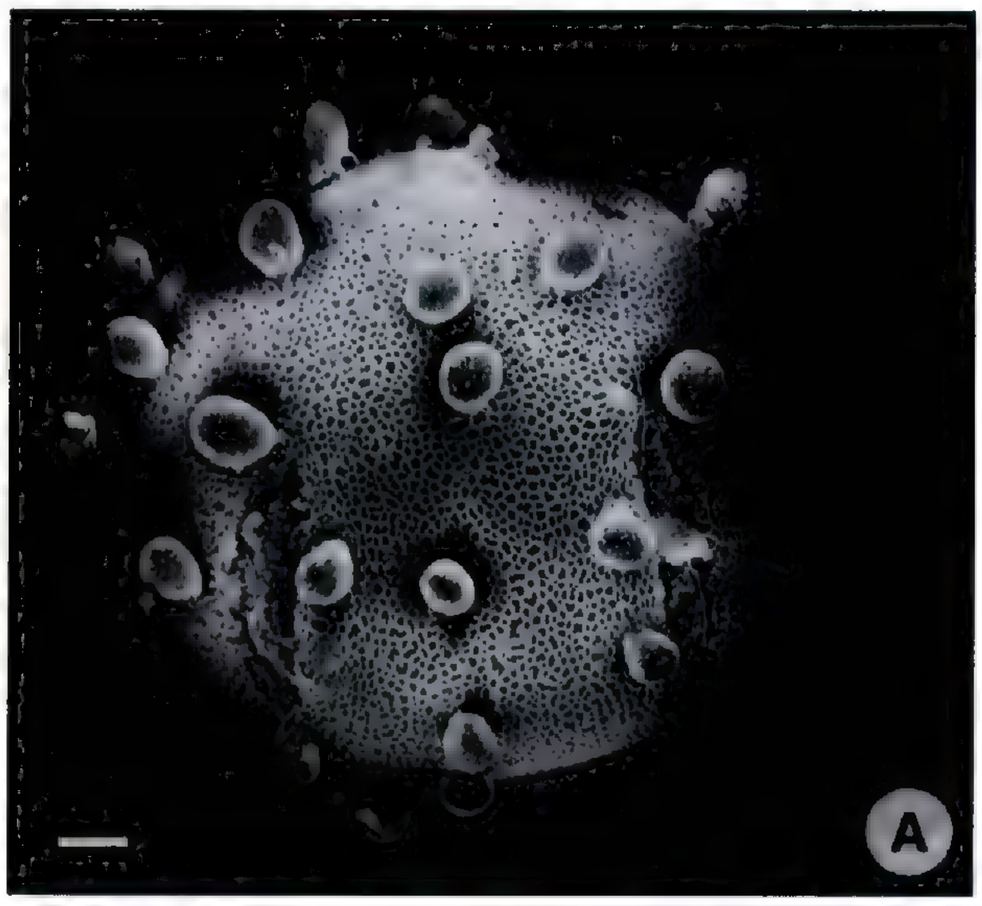
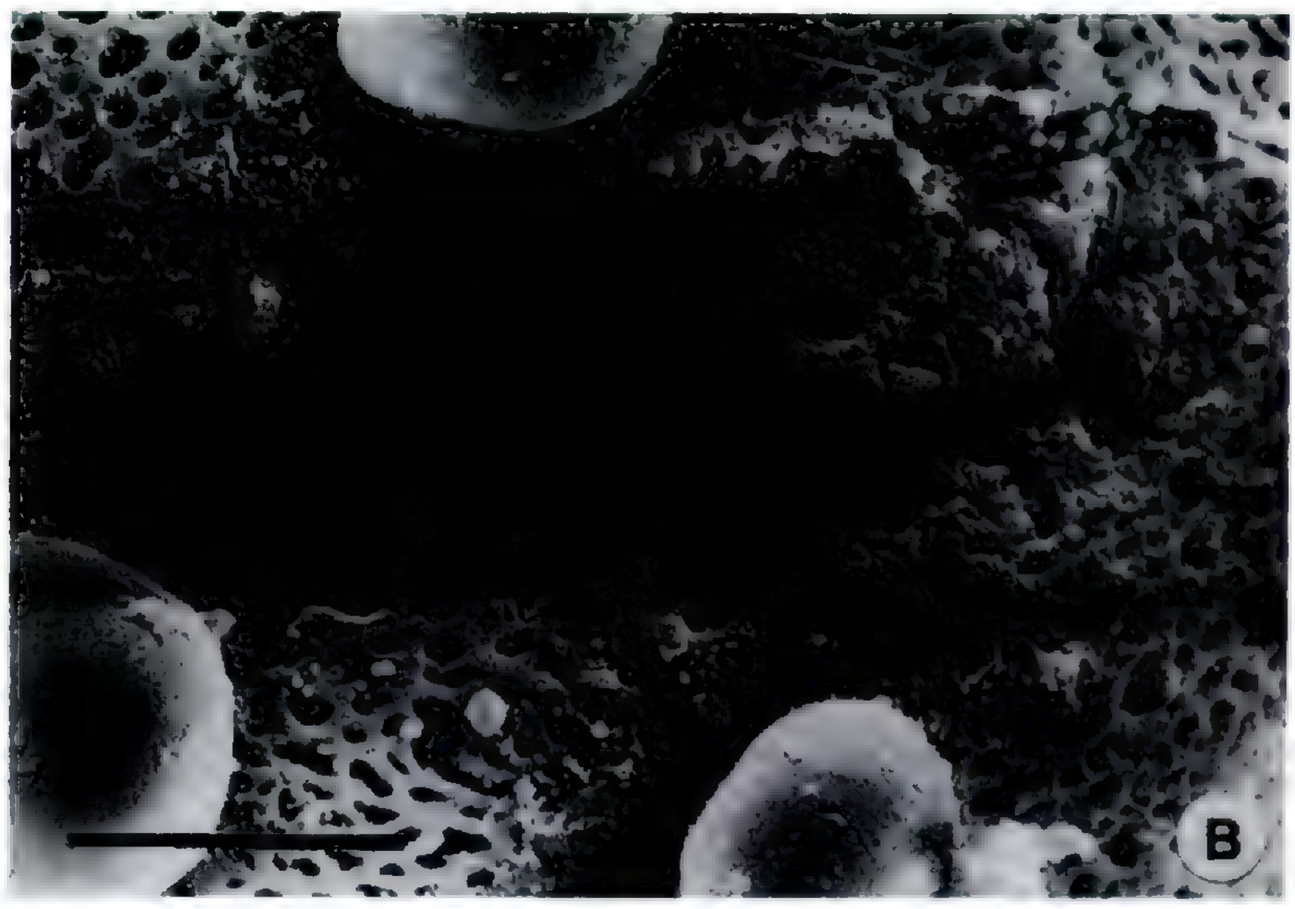
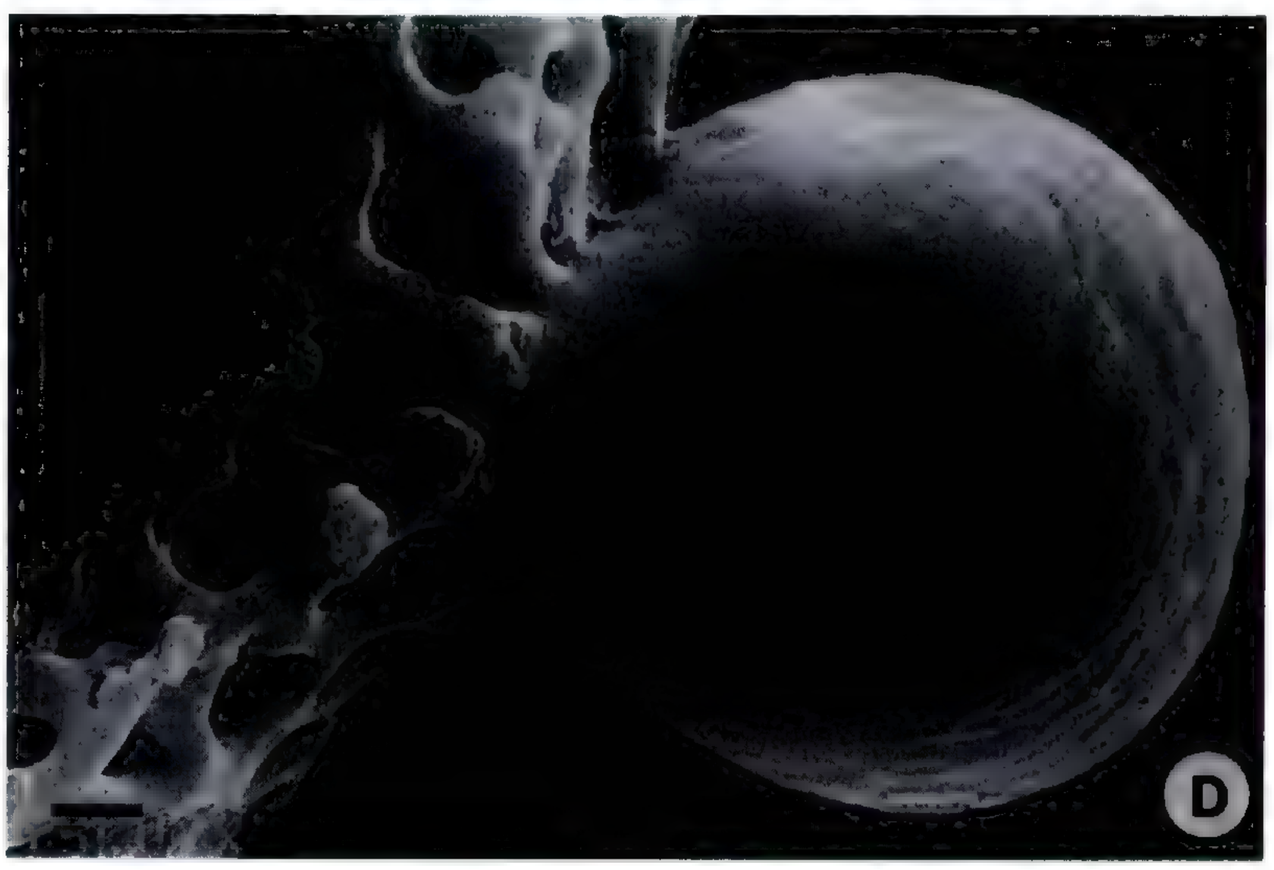


Figura 5. A–G, *Bauhinia mollis* var. *notophila* (*Cabrera et al. 31492*, SI). —A. Grano en vista ecuatorial. —B. Corte de la exina a través de un proceso supratectal. —C. Detalle de la ornamentación de la exina. —D. Detalle del colpo en A. —E. Grano en vista ecuatorial; se observan de 2 colpos. —F y G. Estratificación de la exina en corte delgado: F a través de un colpo. G proceso supratectal gemado. c = colpo; i = intina; n = nexina; s = sexina. Fotomicrografías: A–D: MEB; E–G: MF (E montado según Wodehouse, 1935). Escalas: A, D = 10 μm; B, C = 1 μm; E = 20 μm; F, G = 2 μm.







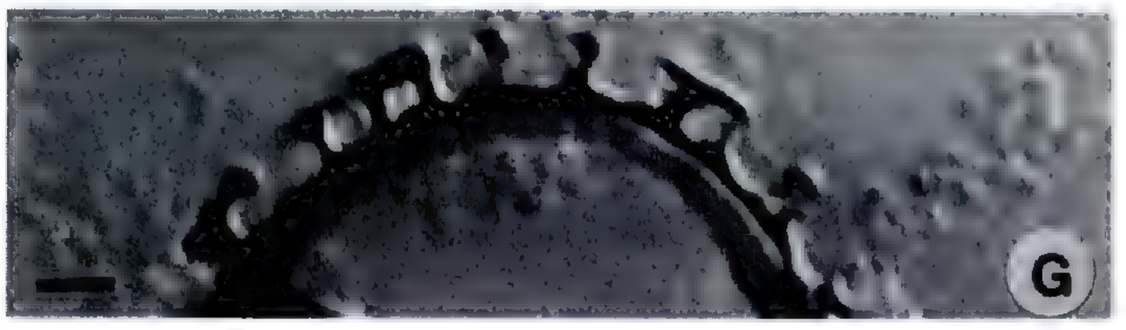


Figura 6. A–G, *Bauhinia forficata* subsp. *pruinosa* (*Schreiter 409*, SI). —A. Grano en vista ecuatorial. —B. Detalle del colpo. —C. Detalle de la ornamentación de la exina (las partículas blancas sobre los muros son impurezas del preparado). —D. Estratificación y ornamentación de la exina fracturada. —E. Corte de la exina. —F y G. (Granos sin acetolizar) Estratificación de la exina en corte delgado. co = columelas; e = exina; i = intina, n = nexina con superficie interna escabrosa; st = semitectum. Fotomicrografías: A–E: MEB; F: MF; G: CI. Escalas: A, B = 10 μm; C–E = 1 μm; F, G = 2 μm.

cortes de los granos, y las medidas de la pared fueron realizadas sobre cortes ópticos observados al microscopio de contraste de interferencia: CI (Fig. 7F).

Material estudiado. ARGENTINA. **Misiones:** Dpto. Gral. M. Belgrano, Bernardo de Irigoyen, naciente del río Pepirí Guazú, 26°15′S, 53°38′W, 19 Nov. 1995, *Gualianone et al. 2953* (BAB, SI). BRASIL. **Paraná:** Guarianaçu-Guaraní, 7 Nov. 1963, *Hatchbach 10374* (SI).

Discusión

Las especies argentinas del género *Bauhinia* están comprendidas dentro de 3 tipos morfológicos diferentes:

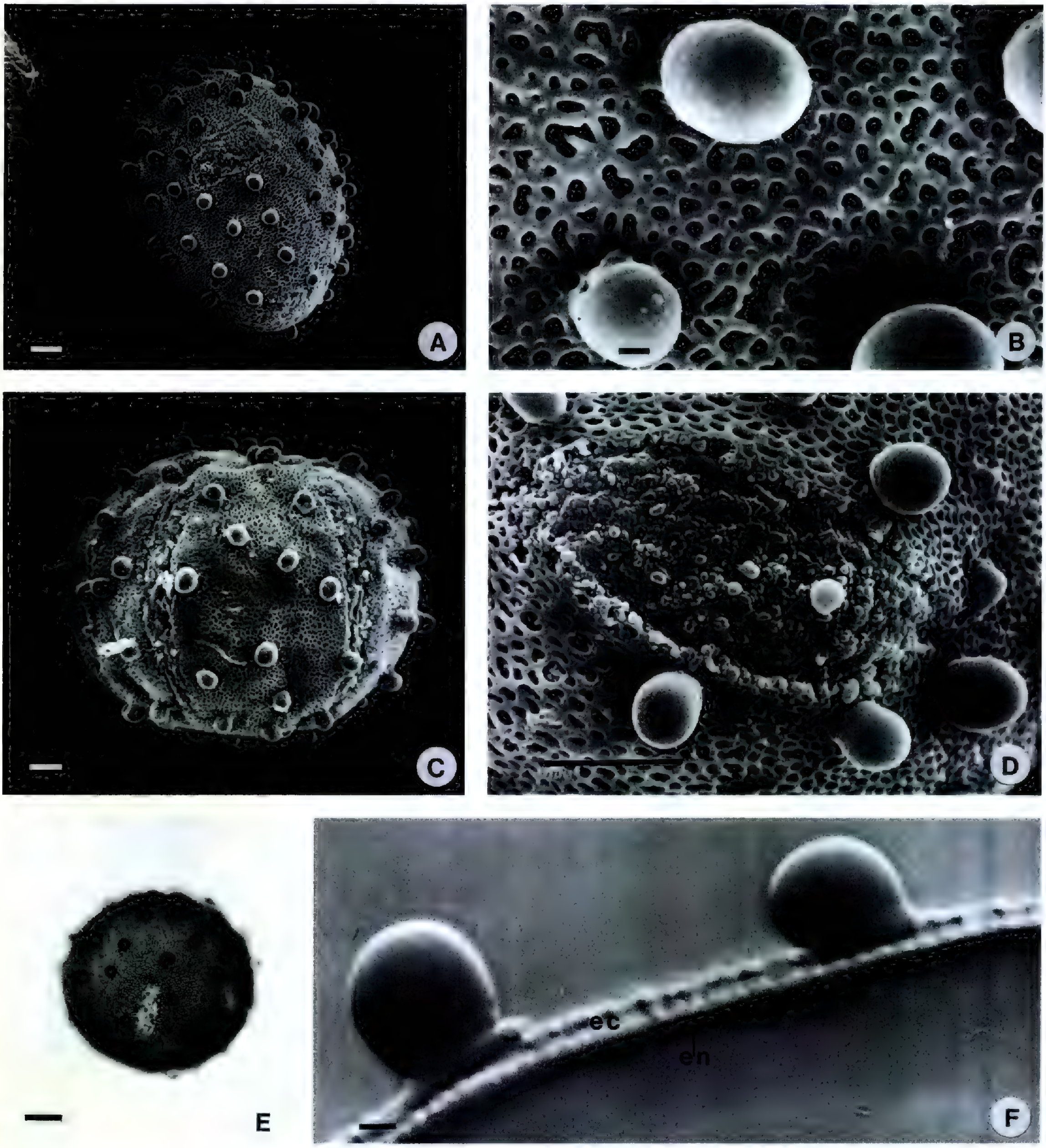


Figura 7. A, B, D, *Bauhinia affinis* (*Guaglianone et al. 2953*, BAB, SI); E, F (*Hatschbach 10374*, SI); C, *Bauhinia hagenbeckii* (*Krapovickas et al. 13920*, SI). —A. Grano en vista ecuatorial. —B. Detalle de la ornamentación de la exina. —C. Grano en vista ecuatorial. —D. Detalle de un colpo. —E. Grano en vista ecuatorial. —F. Estratificación de la exina en corte óptico. ec = ectexina; en = endexina. Fotomicrografías: A–D: MEB; E: MF (montado según Wodehouse, 1935); F: CI, montado en glicerina, hidrato de cloral 1:4. Escalas: A, C, D = 10 μm; B = 1 μm; E = 20 μm; F = 2 μm.

Tipo 1: 3-zonocolporados, tectados, tectum microgranuloso con procesos supratectales esféricos, rugosos.

B. microstachya var. *microstachya*

Tipo 2: 3-zonocolporados a porados, microrreticulados, semitectum con procesos supratectales espiniformes.

B. uruguayensis

Tipo 3: 4–6-zonocolpados, reticulados, semitectum con procesos supratectales gemados.

Subtipo a: Colpos largos y angostos (largo 4 o más veces el ancho), Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial generalmente mayor de 0.17.

B. argentinensis var. *argentinensis*
B. argentinensis var. *megasiphon*
B. bauhinioides

Subtipo b: Colpos relativamente cortos y anchos (largo menor de 4 veces el ancho), Nº de procesos supratectales del amb en vista polar/diámetro ecuatorial generalmente menor de 0.17.

B. mollis var. *notophila*
B. forficata subsp. *pruinosa*
B. affinis

TIPO 1

Al relacionar los tipos morfológicos con las divisiones infragenéricas establecidas por Wunderlin et al. (1987) se observó que *B. microstachya* var. *microstachya* posee polen de forma y estructura similar a otras especies del subg. *Phanera* sect. *Schnella*, por ej.: *B. smilacina* (Schott) Steud. y *B. flexuosa* Moric; asimismo se señala que en dicha sección predominan plantas de hábito trepador (lianas o arbustos escandentes con zarcillos) y frutos indehiscentes.

TIPO 2

Bauhinia uruguayensis fue ubicada erróneamente por Schmitz (1973: 406) en el género *Binaria* Raf. (= subsect. *Binaria* (Raf.) Wunderlin, Larsen & Larsen sect. *Caulotretus* DC. del subg. *Phanera* en Wunderlin et al., 1987); las especies de este grupo son lianas con zarcillos, frutos leñosos, dehiscentes y polen tricolporado, prolato, exina de ornamentación finamente perforada o rugulada a verrucosa y sin procesos supratectales; estos caracteres morfológicos y palinológicos difieren de los presentes en *B. uruguayensis*. Según la morfología polínica aquí descripta y lo observado por Ferguson (1990), esta especie fue correctamente ubicada en la sect. *Benthamia* Fortunato & Wunderlin (Fortunato & Wunderlin, 1985) del subgénero *Elayuna* (Wunderlin et al., 1987), grupo en el que predominan las formas biológicas de árboles y arbustos con espinas, legumbres leñosas, dehiscentes y diferente morfología floral que la existente en la subsect. *Binaria* (Fortunato & Wunderlin, 1985; Vaz, 1979). La equívoca ubicación taxonómica señalada por Schmitz (1973) probablemente se explica porque este autor incluyó en el género *Binaria* a todas las especies citadas por Bentham (1870) en la sect. *Tylotaea* Vogel (= subsect. *Binaria*), sin estudiar el polen, ni relacionarlo con los caracteres macromorfológicos (Cuadro 1). La forma polínica observada en *B. glaziovii* Taub. (especie tipo de la sect. *Benthamia*) es similar a la presente en *B. uruguayensis*, por eso no se entiende sobre que base Schmitz (1973) la consideró en el género *Pauletia* Cav. sub. nom. *P. glaziovii* (Taub.) Schmitz (= sect. *Pauletia* (Cav.) DC. subg. *Bauhinia*), grupo taxonómico que no posee caracteres macromorfológicos ni polínicos relacionados con el subg. *Elayuna* sect. *Benthamia* (Fortunato & Wunderlin, 1985; Fortunato, 1986; Ferguson & Pearce, 1986; Wunderlin et al., 1987; Ferguson, 1990; Cuadro 1).

Asimismo, la morfología del polen de *B. uruguayensis* (subg. *Elayuna*), presenta similitud con lo descripto por Wunderlin et al. (1987) en especies del subg. *Phanera* sect. *Phanera* (*B. involucellata* Kurz, *B. similis* Craib, *B. bracteata* (Grah. ex Benth.) Baker), lianas de Indochina y Malasia, y sect. *Lasiobema* (Korthals) Benth. (*B. harmsiana* Hoss.), liana de Tailandia. Los subg. *Elayuna* y *Phanera* agrupan especies filogenéticamente poco relacionadas, especialmente en el hábito de crecimiento y en la morfología floral; esta falta de parentesco entre las formas polínicas y la macromorfología, también ha sido señalada por Ferguson (1990); la similitud en estos tipos polínicos probablemente esté relacionada con una convergencia de factores adaptativos al tipo de polinizadores presentes en los distintos ecosistemas (Kalin Arroyo, 1981).

TIPO 3

Las especies del subg. *Bauhinia* que crecen en la Argentina fueron consideradas por Wunderlin et al. (1987) en la sect. *Pauletia* (Cav.) DC. Series:

a. *Cansenia* (Raf.) Wunderlin, Larsen & Larsen (= *B. forficata* subsp. *pruinosa*, *B. mollis* var. *notophila* y *B. affinis*).
b. *Perlebia* (Mart.) Wunderlin, Larsen & Larsen (= *B. bauhinioides*).
c. *Pentandra* Wunderlin, Larsen & Larsen (= *B. argentinensis*).

La morfología polínica de todas las especies estudiadas de este tipo posee colpos de forma y tamaño variable. Esta variación presente en los colpos ha sido poco considerada por los distintos autores que describieron el polen del género. Los tres taxa estudiados de la ser. *Cansenia* presentan colpos relativamente cortos y anchos (Figs. 5A, D, E; 6A, B; 7A, D, E), en cambio en las otras dos especies de las series *Perlebia* y *Pentandra* los colpos son largos y angostos (Figs. 3A, C; 4A, B, E). Sin hacer mención de la forma y tamaño de los colpos, Wunderlin et al. (1987) señalan que existe afinidad entre la ser. *Perlebia* y la ser. *Pentandra* y concluyen que la primera serie derivaría de la segunda; esta relación filogenética sería corrobo-

rada por la similitud observada entre la macromorfología y el polen de los representantes estudiados.

Las especies que crecen en Argentina de la ser. *Cansenia* presentan granos 4–6-zonocolpados. Según lo citado por Ferguson y Pearce (1986) en este grupo taxonómico existen subgrupos morfológicos algunos de lo cuales han sido analizados y se encuentran actualmente en estudio para futuras contribuciones.

En otra especie de la ser. *Pentandra*: *B. haagenbeckii* Harms, de Paraguay y Brasil (Fig. 7C) también se ha observado la presencia de colpos largos y angostos, siendo probable que este tipo de apertura tenga validez taxonómica para la caracterización de esta serie; por otro lado, en el material estudiado de esta sección de Argentina, Paraguay y Brasil, se comprueba siempre la presencia de granos colpados, siendo probable que por un error tipográfico en Wunderlin et al. (1987), hayan descripto a la ser. *Pentandra* como 3–7-colporado.

Zhang (1995) al realizar un análisis cladístico de 70 caracteres morfológicos, entre los que evalúa 18 de venación foliar y ninguno de los distintos tipos de polen, considera en parte al subg. *Bauhinia* como un grupo monofilético, pero al referirse al subg. *Elayuna*, incluye en él entre otras secciones a la sect. *Schnella* del subg. *Phanera*, grupo taxonómico que posee caracteres polínicos y morfológicos de diferente afinidad (Cuadro 1).

Conclusiones

La evaluación cladística de los caracteres morfológicos efectuada por Zhang (1995) del género *Bauhinia* considera no naturales algunos de los subgéneros establecidos por Wunderlin et al. (1987), no obstante el presente estudio del polen y su relación con la morfología externa de las especies que crecen en la Argentina coincide en general con este sistema de clasificación (Wunderlin et al., 1987). Asimismo se señala, que el análisis molecular realizado sobre la presencia/ausencia del intron *rpL2* en genomas de cloroplastos (Lai et al., 1997), en parte acepta el agrupamiento y filogenia propuesta por Wunderlin et al. (1987). Sin embargo para una mejor evaluación de las relaciones filogenéticas del género serán necesarios futuros estudios que correlacionen los distintos datos morfológicos con otras metodologías moleculares.

Literatura Citada

Barth, O. M. & C. P. Bouzada. 1964. Catálogo sistemático dos pólens das plantas arbóreas do Brasil meridional. Mem. Inst. Oswaldo Cruz 62: 169–192. lam. I–IV.

Bentham, G. 1870. Leguminosae II, Swartzieae et Caesalpinioideae. *En:* C. F. Martius, Fl. bras. 15(2): 1–254. Ed. F. Fleischer, Lipsiae.

Erdtman, G. 1960. The acelolysis method, a revised description. Sventk Bot. Tidskr. 54: 561–564.

———. 1972. Pollen Morphology and Plant Taxonomy. Angiosperms. Hafner, New York.

Ferguson, I. K. 1990. Common features of pollen exine ornamentation and stratification of some palaeotropical and neotropical *Bauhinia* L. species (Leguminosae: Caesalpinioideae). J. Palynol. 26: 73–82.

——— & K. J. Pearce. 1986. Observations on the pollen morphology of the genus *Bauhinia* L. (Leguminosae: Caesalpinioideae) in the Neotropics. Pp. 283–296 *en* S. Blackmore & I. K. Ferguson (editores), Series 12. Pollen and Spores: Form and Function, Linnean Society Symposium. The Linnean Society of London, London.

Fortunato, R. H. 1984. Nota crítica sobre *Bauhinia mollis* (Bong.) D. Dietr. (Caesalpinioideae, Leguminosae). Parodiana 3: 43–52.

———. 1986. Revisión del género *Bauhinia* (Cercideae, Caesalpinioideae, Fabaceae) para la Argentina. Darwiniana 27(1–4): 527–557.

———. 1996. *Bauhinia affinis* (Fabaceae) una nueva cita para la Flora Argentina. Darwiniana 34: 404–409.

——— & R. Wunderlin. 1985. *Benthamia*: Una nueva sección del género *Bauhinia* L. (Cercideae, Caesalpinioideae, Fabaceae). Parodiana 3: 317–327.

Hideux, M. & L. Marceau. 1972. Techniques d'étude du pollen au MEB: Méthode simple de coupes. Adansonia, Ser. 2, 12: 609–618.

Holmgren, P. K., N. Holmgren & L. C. Barnett. 1990. Index Herbariorum, Part I. The Herbaria of the World, 8 ed. International Association for Plant Taxonomy, New York Botanical Garden, Bronx, New York.

Kalin Arroyo, M. T. 1981. Breeding systems and pollination biology in Leguminosae. Pp. 723–770 *en* R. M. Polhill & P. H. Raven (editores), Advances in Legume Systematics, Vol. 2. Royal Botanic Gardens, Kew.

Lai, M., J. Sceppa, J. A. Ballenger, J. J. Doyle & R. Wunderlin. 1997. Polymorphism for the presence of the *rpL2* intron in chloroplast genomes of *Bauhinia* (Leguminosae). Syst. Bot. 22: 519–528.

Larsen, K. & S. S. Larsen. 1983. The genus *Bauhinia* in Australia. Taxonomy and palynology. Bot. Helv. 93: 213–220.

Larsen, S. S. 1975. Pollen morphology of Thai species of *Bauhinia* (Caesalpiniaceae). Grana 14: 114–131.

Melhem, T. S. & S. L. Salgado-Labouriau. 1963. Pollen grains of plants of the Cerrado. V. Leguminosae–Caesalpinioideae. Rev. Brasil. Biol. 23: 369–387, 50 figs.

Palacios Chavez, R. 1970. Morfología del polen de las especies mexicanas más comunes del género *Bauhinia* (Leguminosae). Anales Esc. Nac. Ci. Biol. 18: 107–136.

Punt, W., S. Blackmore, S. Nilsson & A. Le Thomas. 1994. Glossary of pollen and spore terminology. *En* LPP Contributions Series Nº 1: 1–71. Utrecht, The Netherlands.

Smith, F. G. 1964. Some pollen grains in the Caesalpinioideae of East Africa. Pollen & Spores 6: 85–98.

Schmitz, A. 1973. Contribution palynologique à la taxonomie des *Bauhinieae* (Caesalpiniaceae). Bull. Jard. Bot. Belg. 43: 369–423.

Vaz, A. M. S. da F. 1979. Considerações sobre a taxonomia do gênero *Bauhinia* L. sect. *Tylotaea* Vog. (Le-

guminosae–Caesalpinioideae) do Brasil. Rodriguésia 31(51): 127–234.
Wodehouse, R. H. 1935. Pollen Grains. McGraw-Hill, New York & London.
Wunderlin, R. P., K. Larsen & S. S. Larsen. 1987. Reorganization of the *Cercideae* (Fabaceae: Caesalpinioideae). Kongel. Danske Vidensk. Selsk., Biol. Skr. 28: 1–40.
Zhang, Dian-Xiang. 1995. A cladistic analysis of *Bauhinia* L. (Leguminosae: Cercideae). Chin. J. Bot. 7: 55–64.

STATISTICAL SUMMARY OF SOME OF THE ACTIVITIES IN THE MISSOURI BOTANICAL GARDEN HERBARIUM, 2000

	Vascular	Bryophyte	Total
Acquisition of Specimens			
Staff Collections	24,185	3,346	27,531
Purchase	0	0	0
Exchange	32,211	3,802	36,013
Gifts	8,182	259	8,441
Total acquisitions	64,578	7,407	71,985
Mountings			
Newly mounted	57,246	33,830	91,076
Repairs			
Specimens repaired	21,196	n/a	21,196
Specimens stamped	17,172	n/a	17,172
Total repairs	38,368	0	38,368
Specimens sent			
On exchange	21,867	4,558	26,425
As gifts	15,282	1,278	16,560
Total	37,149	5,836	42,985
Loans sent			
Total transactions	314	42	356
Total specimens	29,385	1,905	31,290
To U.S. institutions			
Transactions	161	31	192
Specimens	16,631	1,028	17,659
To foreign institutions			
Transactions	153	11	164
Specimens	12,754	877	13,631
To student investigators			
Transactions	42	8	50
Specimens	7,423	1,129	8,552
To professional investigators			
Transactions	272	34	306
Specimens	21,962	776	22,738
Loans Received			
Transactions	171	59	230
Specimens	14,968	7,950	22,918

	From U.S.A.	From abroad	Total
Visitors	273	105	378

The total number of mounted, accessioned specimens in the herbarium on 1 January 2001 was 5,110,376 (4,746,428 vascular plants and 363,948 bryophytes).

The Garden's herbarium is closely associated with its database management system, TROPICOS. The charts below summarize some of the statistics from TROPICOS both for the calendar year 2000 and as year-end totals. Note that the specimen records in TROPICOS are primarily based on MO specimens, meaning that about thirty-one percent of the bryophytes (an increase of about seven percent over 1999) and thirty-one percent of the vascular plants (an increase of about two percent) in the herbarium are now computerized, with an overall total of about thirty-one percent (an increase of about three percent).

TROPICOS records—Calendar Year 2000 Additions

	Bryophytes	Vascular Plants	Total
Specimens	33,100	114,124	147,224
Names	2,316	22,334	24,650
Synonyms	1,459	10,027	11,486
Distributions	1,668	27,004	28,672
Types	605	14,420	15,025
Bibliography	1,498	3,024	4,522

TROPICOS records—Year-End 2000 Totals

	Bryophytes	Vascular Plants	Total
Specimens	112,589	1,453,832	1,566,421
Names	99,500	769,948	869,448
Synonyms	62,543	380,145	442,688
Distributions	38,659	767,389	806,048
Types	7,431	259,577	267,008
Bibliography	22,104	61,049	83,153
Specimens in herbarium	363,948	4,746,428	5,110,376
Percent of specimens computerized	31	31	31

In TROPICOS, literature-based Synonymy is always linked to a reference in Bibliography and directly with at least two records in Names, the synonym, often a basionym, and the correct name according to the reference. Additional synonymy may be derived from these direct links, e.g., all other combinations of a basionym treated as a synonym of a given name are also synonyms of it.

—Marshall R. Crosby

NOTICE

The Rupert Barneby Award

The New York Botanical Garden is pleased to announce that Dr. Colin E. Hughes, currently a Royal Society University Research Fellow at the Department of Plant Sciences, University of Oxford, UK, is the recipient of the Rupert Barneby Award for the year 2001. Dr. Hughes will be studying the systematics of Andean *Lupinus* as part of a larger monographic study to establish a new infrageneric classification of the genus and investigate a number of more fundamental biogeographic, domestication, and evolutionary questions.

The New York Botanical Garden now invites applications for the Rupert Barneby Award for the year 2002. The award of U.S. $1,000 is to assist researchers to visit The New York Botanical Garden to study the rich collection of Leguminosae. Anyone interested in applying for the award should submit their curriculum vitae, a detailed letter describing the project for which the award is sought, and the names of two or three referees. Travel to the NYBG should be planned for sometime in the year 2002. The application should be addressed to Dr. James L. Luteyn, Institute of Systematic Botany, The New York Botanical Garden, 200th Street and Kazimiroff Blvd., Bronx, New York 10458-5126 U.S.A., and received no later than December 1, 2001. Announcement of the recipient will be made by December 15th.

The Rupert Barneby Fund for Research in Legume Systematics was established at The New York Botanical Garden in 1991 to honor Rupert by institutionalizing his legacy. The Fund had three purposes specifically chosen to reflect Rupert's wishes: first, to support legume research at The New York Botanical Garden; second, to provide monies to bring legume researchers from around the world to the Garden for extended visits to study the collections Rupert so painstakingly worked to improve; and third, to eventually provide an endowed chair for a legume researcher at the Garden.

Annually since 1991, the Fund has sponsored the competitive "Rupert Barneby Award" in the amount of $1,000 to bring one or more visiting scientists to the Garden for the study of our collection of legumes.

The money for the Fund comes from a variety of sources, but primarily through private gifts. Anyone interested in making a contribution, large or small, to *The Rupert Barneby Fund for Research in Legume Systematics,* which supports this award, may send their check, payable to The New York Botanical Garden, to Dr. James L. Luteyn, The New York Botanical Garden, 200th Street & Kazimiroff Blvd., Bronx, NY 10458-5126, U.S.A.

The New York Botanical Garden is a not-for-profit, tax-exempt organization. Each gift to *The Rupert Barneby Fund for Research in Legume Systematics* will be duly acknowledged by the Garden for its intent and for tax purposes.

Volume 88, Number 1, pp. 1–162 of the ANNALS OF THE MISSOURI BOTANICAL GARDEN was published on April 27, 2001.

Missouri Botanical Garden Library
3 1753 00298 5924

ANNALS OF THE MISSOURI BOTANICAL GARDEN
AND MISSOURI BOTANICAL GARDEN ANNUAL REPORT
ARE NOW AVAILABLE IN JSTOR!

JSTOR®, a not-for-profit organization, is an important endeavor dedicated to helping the scholarly community take advantage of advances in electronic technologies. The JSTOR Archive makes available the complete backfiles of *Annals of The Missouri Botanical Garden, Missouri Botanical Garden Annual Report (1890-1912)*, and more than 100 other scholarly journals to researchers through participating libraries.

JSTOR has recently announced the release of its Ecology & Botany Collection. Developed in cooperation with the Ecological Society of America, the Collection includes the archives of twenty-nine journals, offering online access to more than 1 million pages of important historic literature.

The Ecology & Botany Collection

American Journal of Botany
American Midland Naturalist
American Naturalist
Annals of the Missouri Botanical Garden
Annual Review of Ecology and Systematics
Biotropica
Brittonia
Conservation Biology
Diversity and Distributions
Ecological Applications
Ecological Monographs
Ecology
Evolution
Functional Ecology
Global Ecology and Biogeography Letters
International Journal of Plant Sciences
Journal of Animal Ecology
Journal of Applied Ecology
Journal of Biogeography
Journal of Ecology
Journal of Tropical Ecology
Journal of The Torrey Botanical Society
Limnology and Oceanography
Missouri Botanical Garden Annual Report
New Phytologist
Paleobiology
Quarterly Review of Biology
Systematic Biology
Systematic Botany

Information regarding JSTOR is available at http://www.jstor.org
120 Fifth Avenue, New York, NY 10011

CONTENTS

Coevolution, the 16th Annual Systematics Symposium of the Missouri Botanical Garden
Coevolution: Biological Observations from Yucca Moths to Molecules, An Introduction
Victoria C. Hollowell & P. Mick Richardson 1

Ectomycorrhizae: Co-Evolution, Significance, and Biogeography
Roy E. Halling 5

Multigene Phylogeny of *Epichloë* Species, Fungal Symbionts of Grasses
K. D. Craven, P. T. W. Hsiau, A. Leuchtmann, W. Hollin & C. L. Schardl 14

The Coevolution of Bacterial Endosymbionts and Phloem-feeding Insects
Nancy A. Moran 35

Chemical Mediation of Coevolution: Phylogenetic Evidence for Apiaceae and Associates
May R. Berenbaum 45

The Genus *Striga* (Scrophulariaceae) in Africa
Kamal I. Mohamed, Lytton John Musselman & Charles R. Riches 60

Phylogeny and Historical Biogeography of Lauraceae: Evidence from the Chloroplast and Nuclear Genomes
André S. Chanderbali, Henk van der Werff & Susanne S. Renner 104

The Phylogenetic Relationships of *Lembocarpus* and *Goyazia* (Gesneriaceae) Based on *ndh*F Sequences
James F. Smith 135

Morfología del Polen de las Especies Argentinas de *Bauhinia* (Cercideae, Caesalpinioideae, Fabaceae)
Juan C. Gamerro y Renée H. Fortunato 144

Statistical Summary of Some of the Activities in the Missouri Botanical Garden Herbarium, 2000
Marshall R. Crosby 159

Notice 161

Cover illustration. Top: *Pronuba yuccasella*, female, in the act of gathering pollen from the anthers of *Yucca filamentosa*. Bottom: Pistils of *Y. filamentosa*, showing, from left, a deformed specimen and varying lengths of the stamens. From *Annual Rep. Missouri Bot. Gard.* 1892, The Yucca Moth and Yucca Pollination, by C. V. Riley. See Coevolution, the 16th Annual Systematics Symposium of the Missouri Botanical Garden: Introduction, first issue of this volume.

QK1
.A475
c.1

Annals of the Missouri Botanical Garden

2001

Volume 88
Number 2

Volume 88, Number 2
Spring 2001

Annals of the
Missouri Botanical Garden

The Annals, published quarterly, contains papers, primarily in systematic botany, contributed from the Missouri Botanical Garden, St. Louis. Papers originating outside the Garden will also be accepted. All manuscripts are peer-reviewed by qualified, independent reviewers. Authors should write the Managing Editor for information concerning arrangements for publishing in the ANNALS. Instructions to Authors are printed in the back of the last issue of each volume and are also available online at www.mobot.org/mbgpress.

Editorial Committee

Victoria C. Hollowell
Editor,
Missouri Botanical Garden

Amy Scheuler McPherson
Managing Editor,
Missouri Botanical Garden

Diana Gunter
Associate Editor,
Missouri Botanical Garden

Aida Kadunic
Senior Secretary

Barbara Mack
Administrative Assistant

Ihsan A. Al-Shehbaz
Missouri Botanical Garden

Gerrit Davidse
Missouri Botanical Garden

Roy E. Gereau
Missouri Botanical Garden

Peter Goldblatt
Missouri Botanical Garden

Gordon McPherson
Missouri Botanical Garden

P. Mick Richardson
Missouri Botanical Garden

Henk van der Werff
Missouri Botanical Garden

For subscription information contact ANNALS OF THE MISSOURI GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897. Subscription price for 2001 is $135 per volume U.S., $145 Canada & Mexico, $170 all other countries. Four issues per volume. The journal *Novon* is included in the subscription price of the ANNALS.

annals@mobot.org (editorial queries)
http://www.mobot.org/mbgpress

© Missouri Botanical Garden 2001

The mission of the Missouri Botanical Garden is to discover and share knowledge about plants and their environment, in order to preserve and enrich life.

♾ This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

THE ANNALS OF THE MISSOURI BOTANICAL GARDEN (ISSN 0026-6493) is published quarterly by the Missouri Botanical Garden, 2345 Tower Grove Avenue, St. Louis, MO 63110. Periodicals postage paid at St. Louis, MO and additional mailing offices. POSTMASTER: Send address changes to ANNALS OF THE MISSOURI BOTANICAL GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897.

Volume 88
Number 2
2001

Annals
of the
Missouri
Botanical
Garden

PHYLOGENETIC ANALYSIS OF ASTERIDS BASED ON SEQUENCES OF FOUR GENES[1]

Dirk C. Albach,[2,3] *Pamela S. Soltis,*[2] *Douglas E. Soltis,*[2] *and Richard G. Olmstead*[4]

ABSTRACT

Asterids, comprising about one-third of all angiosperm species, include almost all species that produce iridoids and that have sympetalous corollas, and most species that have unitegmic-tenuinucellate ovules. To elucidate their phylogeny, we used separate and combined data sets of sequences from 18S rDNA, *rbc*L, *ndh*F, and *atp*B, with a maximum of 7168 base pairs for 158 genera, for phylogenetic analysis. The analyses resolved the major lineages of asterids s.l., confirming the feasibility of analyses of large data sets in phylogenetic systematics. Most of the taxa fall within one of four major clades. Cornales, comprising Cornaceae, Nyssaceae, Hydrangeaceae, Loasaceae, and Hydrostachyaceae, are sister to the remaining genera. Ericales consist of taxa traditionally included in Ericales, Primulales, Ebenales, and Theales, as well as Polemoniaceae and Balsaminaceae. They are sister to euasterids I and euasterids II. The euasterids I include Lamiales, Boraginaceae, Solanales, and Gentianales. The relationship of Boraginaceae as sister to Lamiales, even though weakly supported, is reported for the first time. The euasterids II comprise Asterales, Apiales, Dipsacales, *Escallonia*, *Eremosyne*, and *Berzelia*, and Aquifoliales. Dipsacales, including *Sambucus* and *Viburnum*, are monophyletic and sister to Apiales. The data set of 158 taxa sequenced for four genes gives us insight into both the evolution of the asterids and the molecular evolution of the four genes. Some striking differences in rates and patterns of molecular evolution appear both within and among the genes; for example, *ndh*F consists of two very different regions with the second region apparently evolving under reduced selection pressure. The four genes differ in their base composition, transition-transversion bias, and in their most common substitutions.

Key words: 18S rDNA, asterids, *atp*B, Cornales, Ericales, *ndh*F, *rbc*L.

For over a hundred years, biologists have tried to classify organisms to reflect their evolutionary history. Inferring evolutionary history among higher categories of angiosperms, however, has been par-

[1] This research was supported in part by NSF grants DEB-9707868 to PSS and DES, and DBI-9512890 to PSS and DES. We thank L. Hufford, K. Bremer, P. M. Richardson, and two anonymous reviewers for helpful comments on the manuscript, and D. Swofford for access to test versions of PAUP *4.0. We also thank M. Axtell and S. Swensen (Ithaca College), G. Plunkett (Virginia Commonwealth University), Q.-Y. Xiang (Idaho State University), L. Johnson (North Carolina State University), B. and K. Bremer (Uppsala University), J. Smith (Boise State University), M. W. Chase (Royal Botanical Gardens, Kew), C. Morton (Auburn University), R. Jansen (University of Texas), T. Motley and L. Struwe (New York Botanical Garden), B. Tankersley (formerly Missouri Botanical Garden, now Chicago Botanic Garden), D. Bar-Zvi (Fairchild Tropical Garden), T. Omar (Seattle Arboretum), and D. Ewing (University of Washington Greenhouses), who shared plant material, DNA, and sequences.

[2] Department of Botany, Washington State University, Pullman, Washington 99164-4238, U.S.A.

[3] Current address: Botanisches Institut der Universität Wien, Rennweg 14, 1030 Wien, Austria.

[4] Department of Botany, University of Washington, Seattle, Washington 95195-5325, U.S.A.

ticularly difficult where distinctive characters may have been obscured by subsequent evolution. One of the early higher categories of angiosperms to be recognized was the Monopetalae (de Jussieu, 1789), which included plants with fused petals. The concept of Monopetalae, later called Sympetalae (Reichenbach, 1828), has changed considerably over time (see review by Wagenitz, 1992). Warming (1879) split the Sympetalae into Pentacyclicae, with two series of stamens, and Tetracyclicae, with only one series of stamens. The latter have more recently been referred to as subclass Asteridae (Takhtajan, 1964). The rise of cladistic theory and use of molecular data have led to a reevaluation of the limits and component families of Takhtajan's (1964, 1980, 1997) and Cronquist's (1981) Asteridae. Phylogenetic analyses using morphological data (Hufford, 1992), *rbc*L sequence data (Olmstead et al., 1992, 1993; Chase et al., 1993), and cpDNA restriction site analysis (Downie & Palmer, 1992) showed that the Asteridae are not monophyletic as traditionally circumscribed. A monophyletic Asteridae contains Cronquist's (1981) Asteridae, plus parts of his Hamamelidae (Eucommiales), Dilleniidae (most Theales, Lecythidales, Ericales, Diapensiales, Ebenales, Primulales, Sarraceniaceae, Fouquieriaceae, Loasaceae), and Rosidae (Pittosporaceae, Hydrangeaceae, Byblidaceae, Columelliaceae, Alseuosmiaceae, *Escallonia* and *Montinia* from Grossulariaceae, *Eremosyne* and *Vahlia* from Saxifragaceae, Cornales, Aquifoliaceae, Icacinaceae, Balsaminaceae, and Apiales).

These expanded Asteridae (the asterids sensu the Angiosperm Phylogeny Group (APG), 1998) comprise approximately one-third of all angiosperm species, with almost 80,000 species in about 4700 genera and 100 families (Thorne, 1992). Many species of the asterids contain classes of chemical compounds that are not found outside the clade. For example, almost all species that contain iridoids (Jensen, 1992) and tropane alkaloids (Romeike, 1978) and most of those that produce caffeic acid (Mølgaard, 1985) belong to the asterids. Such chemical compounds have frequently been used as important characters in classification (e.g., Dahlgren et al., 1981). Dahlgren (1975) noted that the occurrence of iridoids highly correlates with sympetalous flowers and embryological characters such as unitegmic-tenuinucellate ovules and cellular endosperm development. However, no one has been able to infer the exact pattern of diversification for these characters because a broadly reconstructed phylogenetic tree has been lacking.

Despite these correlated characters in many asterids, a circumscription of a monophyletic Asteridae has nevertheless been difficult using morphological data alone because of parallelisms (Hufford, 1992) and losses of characters (Olmstead et al., 1992). However, phylogenetic analyses of DNA sequences from the chloroplast genes *rbc*L, *atp*B, and *ndh*F have shown the monophyly of the asterids (Chase et al., 1993; Olmstead et al., 1992, 1993; Chase & Cox, pers. comm.). Analyses of 18S rDNA sequence data were ambiguous, with one set of trees showing asterids to be monophyletic (D. Soltis & P. Soltis, 1997) and another set nesting the Caryophyllidae (Caryophyllales sensu APG, 1998) within them (Soltis et al., 1997a). Furthermore, the interfamilial relationships remain partly unresolved due to the lack of sufficient characters provided by *rbc*L (e.g., Olmstead et al., 1993). Additionally, several critical taxa have been left out of previous analyses. The circumscription and interrelationships among components of asterids, therefore, remain unclear.

A further problem in reconstructing the phylogeny of the asterids is the sheer scope of the analysis. Until recently, phylogenetic analyses of groups as large as asterids were plagued by several limitations, including slow computation times (and thus inadequate searching of tree space), an insufficient number of characters to resolve the number of taxa, and insufficient sampling of taxa. However, large-scale phylogenetic analyses are now considered much more tractable, due to the development of new approaches to phylogeny reconstruction (e.g., Mishler et al., 1994; P. Soltis & D. Soltis, 1996; Rice et al., 1997), new and faster computer programs (e.g., "parsimony-jackknifing" Farris et al., 1996; PAUP*4.0, Swofford, 1998; the RATCHET, K. Nixon, 1999), simulation reports (Hillis, 1996; Graybeal, 1998; Poe & Swofford, 1999), and empirical studies (e.g., Soltis et al., 1998; Wolf et al., 1998).

Increasing taxa number increases accuracy of a phylogenetic analysis (Hillis, 1996; Graybeal, 1998; Yang, 1998; but see Poe & Swofford, 1999), but it also makes the analysis computationally much more difficult. Even more important in increasing accuracy and resolution of a phylogenetic analysis seems to be adding more characters (Hillis, 1996; Poe & Swofford, 1999; but see Naylor & Brown, 1998), reducing the time for data analysis, and increasing support for clades retrieved (Soltis et al., 1997b, 1998). Therefore, the strategy has been to combine a large number of molecular characters from four genes for a variety of taxa assuring a balanced representation of all major lineages of

asterids. The existence of large data sets for 18S rDNA (e.g., Soltis et al., 1997a), *rbc*L (e.g., Olmstead et al., 1993), *ndh*F (e.g., Olmstead et al., 2000), and *atp*B (e.g., Savolainen et al., 2000), as well as rapid sequence generation by automated means, enable a combined analysis of the asterids s.l. with 7168 characters, 2692 of them parsimony-informative.

18S rDNA and rRNA sequences have been used for a wide range of phylogenetic studies in animals, fungi, protozoa, bacteria, algae, bryophytes, and tracheophytes (e.g., Hamby & Zimmer, 1992; Soltis et al., 1997a). 18S rDNA is 20 percent longer than chloroplast genes *rbc*L (1807 bp versus 1431 bp, Nickrent & Soltis, 1995), but evolves more slowly than *rbc*L, *ndh*F, or *atp*B (Nickrent & Soltis, 1995; Olmstead & Reeves, 1995; D. Soltis & P. Soltis, 1997), and its pattern of evolution is constrained somewhat by its secondary structure (P. Soltis & D. Soltis, 1998). Phylogenies inferred from 18S rDNA, *rbc*L, or *atp*B sequences appear reasonably concordant across angiosperms (Soltis et al., 1997a; Savolainen et al., 2000). Hybridization and introgression are not an issue on this taxonomic level (Chase et al., 1993), and therefore data from these three genes have been combined to analyze major lineages of angiosperms (Soltis et al., 1997b, 1998; Soltis et al., 2000).

The most widely used gene for phylogenetic analyses among angiosperms is *rbc*L (e.g., Chase et al., 1993; Morgan & Soltis, 1993; Olmstead et al., 1993; Xiang et al., 1993). More than 2500 *rbc*L sequences have been generated for angiosperms alone (Källersjö et al., 1998). This chloroplast gene has been applied at the ordinal level (Chase et al., 1993) to studies resolving species relationships (Xiang et al., 1993).

Of the four genes considered herein, the *ndh*F gene evolves fastest with a substitution rate twice that of *rbc*L (Olmstead & Sweere, 1994). *ndh*F is a long chloroplast gene of 2223 bp in tobacco, but its length varies among taxa. Comparative *ndh*F sequencing has been used for intrafamilial studies (e.g., Olmstead & Sweere, 1994; Kim & Jansen, 1995), but rarely above the family level as herein.

Comparative sequencing of the chloroplast *atp*B gene has seen less phylogenetic application than the other three genes. It has been used in studies investigating generic relationships (Hoot et al., 1995) to an analysis of the angiosperms as a whole (Savolainen et al., 2000). A broad analysis using over 500 *atp*B, *rbc*L, and 18S rDNA sequences for angiosperms (Soltis et al., 2000) has further demonstrated the usefulness of *atp*B sequences. At 1497 bp long, *atp*B has a rate of evolution comparable to *rbc*L (Hoot et al., 1995) with its range of application assumed similar.

This study reports more than 200 new sequences of 18S rDNA, *rbc*L, *ndh*F, and *atp*B for the asterids and presents the results of analyses of these four genes singly and in combination. We use these data and analyses to discuss relationships within asterids. This study also compares patterns of molecular evolution with matching data sets for these four different genes.

Materials and Methods

Species Sampled and Sources of Plant Material

The species included in the analyses are given in Appendix 1, along with GenBank accession numbers. Sequences that have not been previously published are given in Appendix 2, along with collection and voucher information.

The emphasis in acquiring new sequences was on families and clades that were not as well represented and on taxa that were thought to subdivide long branches (Hillis, 1998; Graybeal, 1998). We attempted to use the same source DNA as previously used (Olmstead et al., 1993; Olmstead et al., 2000). Where not possible, the same species or genus was used, except in the following cases. *Coriandrum* (*rbc*L, *ndh*F), *Lomatium* (18S rDNA), and *Apium* (*atp*B) were combined for Apiaceae–Apioideae, and *Streptocarpus* and *Saintpaulia* (*atp*B) for Gesneriaceae–Didymocarpeae. *Lamium* and *Ajuga* (*ndh*F) were paired as representatives of gynobasic-styled Lamiaceae, *Loasa* and *Caiophora* (*rbc*L) in Loasaceae, as were *Ardisia* and *Maesa* (*atp*B) for Myrsinaceae. Combining *Ardisia* and *Maesa* may obscure relationships of Myrsinaceae based on results from Anderberg et al. (1998), but these results became known to us too late for this study. *Anagallis* and *Primula* (18S rDNA) were combined for Primulaceae, and *Ixora* and *Coffea* (*atp*B) for Rubiaceae–Ixoroideae. *Theretia* (*rbc*L, *ndh*F), *Tabernaemontana* (18S), and *Plumeria* (*atp*B) were combined for Apocynaceae–Plumerioideae. *Alstonia* was included in the 18S rDNA/*rbc*L analysis to represent Apocynaceae–Plumerioideae, but seems sister to the remaining Apocynaceae based on molecular and morphological data (cf. Sennblad & Bremer, 1996). In the outgroup, *Tetracera* and *Dillenia* (*rbc*L) were combined for Dilleniaceae.

The sister group of the asterids is unclear, making the selection of an appropriate outgroup difficult. Both the *rbc*L analysis of angiosperms (Chase et al., 1993) and the 18S rDNA analysis of angiosperms (Soltis et al., 1997a) revealed a large rosid clade

as sister to asterids, so most outgroups were selected from this clade. The asterids and rosids are the two largest clades of core eudicots in an analysis of *rbc*L, *atp*B, and 18S rDNA sequences (Soltis et al., 2000). The outgroup herein consisted of 25 taxa for 18S rDNA and *rbc*L, and 17 taxa for *ndh*F, *atp*B, and the combined analysis of all four genes. Those outgroup taxa used only in the 18S rDNA and *rbc*L data sets are marked with an asterisk in the following list. The outgroups cover all major clades of rosids: nitrogen-fixing clade (*Rhamnus, Quercus*) and *Oxalis* of eurosids I (APG, 1998), Brassicales (*Tropaeolum, Limnanthes**, *Akania**) and Sapindales (*Acer, Staphylea*) of eurosids II, and Dilleniaceae. Dilleniaceae and Caryophyllales (*Plumbago, Phytolacca, Spinacia**) were nested within asterids in the analysis of 18S rDNA (Soltis et al., 1997a). Based on analyses of *rbc*L, 18S rDNA, and *atp*B (Soltis et al., 2000), the Saxifragales (*Heuchera, Cercidiphyllum, Paeonia, Itea**, *Saxifraga**, *Liquidambar**) occur as sister to all other rosids. Representing the early-diverging eudicots are *Sabia, Trochodendron,** and a clade of Ranunculales (*Dicentra, Ranunculus**). The most distantly related outgroup taxa used in these analyses are *Liriodendron* and *Ceratophyllum*, from the grade at the base of the angiosperm tree.

Plant material was obtained from field collections, botanical gardens, or herbarium specimens (Appendix 2). Genomic DNA was extracted from fresh leaf tissue using the CTAB method of Doyle and Doyle (1987) or as modified by Cullings (1992) for herbarium specimens and material dried in silica gel.

AMPLIFICATION AND SEQUENCING

PCR amplifications used 50 pmol of each primer, 10 µl *Taq* DNA polymerase buffer (20 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 10% Tween-20), 1 mM dNTPs, 2.5 units of *Taq* DNA polymerase (Promega and Sigma), and a varying amount of DNA in 100 µl total volume. For amplification of 18S rDNA, 5 µl dimethylsulfoxide (DMSO) was added to facilitate denaturation (Varadaraj & Skinner, 1994).

18S rDNA was amplified using the primers NS1 and C18L*, as given in Bult et al. (1992). *rbc*L genes were amplified using primers Z1 (Zurawski, DNAX Research Institute) and 3′ *rbc*L-primer (Olmstead et al., 1992). Reaction conditions were: premelt at 97°C for 2 min.; 30 cycles of denaturing at 94°C for 1 min., annealing at 48°C for 2 min., and extension at 72°C for 2 min.; plus a final extension step at 72° for 16 min. *ndh*F was amplified using primers 1 and 2110R (Olmstead & Sweere, 1994). Reaction conditions differed by premelt at 94°C for 3 min.; 30 cycles with each cycle as for 18S rDNA; but annealing step at 48° of only 1 min. *atp*B was amplified using primers S2 and S1494R (Hoot et al., 1995). Reaction conditions were: premelt at 94°C for 3 min.; 30 cycles of denaturing at 94°C for 1 min., annealing at 51°C for 1 min., and extension at 72°C for 3 min.; plus a final extension step at 72° for 16 min..

Double-stranded PCR products were sequenced, using the PRISM system (Applied Biosystems, Inc.) following the manufacturer's protocol but using ¼ reactions rather than full, and using both internal primers and those used for amplification, sequencing both strands at almost all positions. Sequencing primers used for 18S rDNA were combinations of NS1, C18E, 626r, 854r, C18H, N18J, 1525r, and C18L; for *rbc*L 1, 674, 1204, 3′, 1351r, 674r; for *ndh*F 1, 536, 972, 1318, 2110r, 1318r, 972r, 536r; for *atp*B S2, S335, S611, S1022, S1494r, S1186r, S766r, S385r. Sequencing reactions were electrophoresed and recorded using an ABI 377 DNA sequencer following the manufacturer's protocol.

ALIGNMENT

DNA sequences were assembled and edited using Sequencher 3.0 (Gene Codes Corp., Inc.). *rbc*L and *atp*B did not show length mutation, except for a two-codon deletion beginning at position 163 in the *atp*B sequences of *Ipomoea* and *Convolvulus*. Only the first 1428 bp of *rbc*L were used, as in previous studies (Chase et al., 1993; Olmstead et al., 1993). The aligned 18S rDNA sequences from asterids were 1855 bp long. Bases 1–41 and 1799–1855 were excluded from the phylogenetic analysis, because the extreme 5′ and 3′ ends were too difficult to read in many taxa. Most length mutations in 18S rDNA are due to indels of a single base pair, confined to a few small regions (Soltis et al., 1997a; P. Soltis & D. Soltis, 1998). Only one small region (of four noted by Soltis et al., 1997a) could not be aligned in asterids. This single region of 18S rDNA that could not be aligned contained seven base pairs corresponding to positions 666–672 on the sequence of *Glycine max*. This one highly variable region corresponds to the terminus of helix E23-1 and was subsequently eliminated from phylogenetic analysis (Swofford & Olsen, 1990).

Alignment of *ndh*F sequences was also done by eye, but 69 indels made alignment more difficult than for the other genes. Six indels were insertions of direct repeats of the preceding codon or codons. Ten of the 69 indels occur in members of the out-

group. Furthermore, 26 indels are restricted to single taxa in this data set (Appendix 3). Sequence length varied between 2151 bp for the Convolvulaceae and 2256 bp for *Eremosyne* with gapped positions treated as missing data.

PHYLOGENETIC ANALYSIS

Data sets as large as those analyzed in this study pose some special problems. When parsimony is employed, only heuristic searches are feasible at all, and even these are computationally so expensive that the globally shortest tree may not be found (Rice et al., 1997). Measures were therefore taken to explore as much tree space as possible (P. Soltis & D. Soltis, 1996; Soltis et al., 1997b).

Seven phylogenetic analyses were conducted using a test version (d60) of the program PAUP* vers. 4.0 (D. L. Swofford, with permission): one for each gene separately, one with a combined 18S rDNA/*rbc*L data set (178 taxa), one with a combined data set of all four genes (137 taxa), and one with a combined data set of taxa that have been sequenced for all four genes plus 21 additional taxa that have been sequenced for three genes only (158 taxa). For the final four-gene analysis, the missing gene was replaced by question marks in this analysis. Those 21 taxa incomplete for one sequence include *Roridula* (18S); *Phyla* (*rbc*L); *Byblis, Aucuba, Eucommia, Roussea, Cobaea, Manilkara, Cyrilla, Sarracenia,* and *Symplocos* (*ndh*F); *Davidia, Barnadesia, Lonicera, Scaevola, Phacelia, Anthocleista, Aeschynanthus, Plantago, Callicarpa,* and *Lantana* (*atp*B).

The search strategy followed D. Soltis and P. Soltis (1997). Five hundred consecutive heuristic searches with random taxon addition and NNI branch-swapping were conducted, with five trees saved per replicate, to increase chances of finding multiple islands of most-parsimonious trees (Maddison, 1991). The trees from the four best replicates were used as starting trees for heuristic searches conducted using MULPARS and TBR branch swapping. Implementing regular bootstrap (Felsenstein, 1985) is impractical with data sets of this size. Support was estimated, therefore, using 500 replicates of the "fast" bootstrap option in PAUP* 4.0d60. Fast bootstrap analyses provide values similar to or slightly lower than those derived from bootstrapping with branch swapping (Mort et al., 2000).

RESULTS

18S rDNA

Of all 1749 sites included in the analysis, 284 sites were parsimony-informative, with 242 informative within asterids. Analysis did not run to completion and was ambiguously interrupted when 5000 trees of one length were found. Shortest trees had 2321 steps (CI = 0.275; RI = 0.593). The strict consensus tree is shown in Figure 4.

Table 1. Changes among stem bases of 18S rDNA at parsimony-informative sites (total number 768 changes at 284 sites).

	To			
From	A	C	G	T
A	—	4	36	2
C	34	—	12	267
G	155	12	—	70
T	26	136	14	—

18S rRNA has a highly conserved secondary structure across angiosperms, and this is reflected by the low number of informative sites per total number of nucleotides. The secondary rRNA structure consists of "stems," areas of paired nucleotides, and "loops" (including bulges of one or more nucleotides), where nucleotides do not pair. Stem bases were identified using the known secondary structure of 18S rDNA in *Glycine max* (Nickrent in Soltis et al., 1997) as a model. In total, 1108 bp (60%) of all 18S rDNA base pairs are found in stem regions. Of the 242 parsimony-informative characters within asterids, 133 (55%) are stem bases. This number resembles closely the numbers found in other studies (58% across all angiosperms, Soltis et al., 1997a; 57.7% in Polemoniaceae, Johnson et al., 1999; 54% in a comparison of *Glycine* and *Phoradendron* (Viscaceae), Nickrent & Franchina, 1990). In animal ribosomal DNA, stem bases and loop bases have about the same probability of being parsimony-informative (12% and 14%, respectively), as found by Vawter and Brown (1993).

As stem bases pair, a base change might prevent pairing, therefore favoring a compensatory change on the opposite strand. Another kind of compensatory change involves uracil, which can pair with either guanine or adenine (U-G ⇔ U-A). Most transitions among stem bases, therefore, retain base-pairing in stems. Mutations in stem bases at parsimony-informative sites were estimated using the TRACE option in MacClade 3.04 (Maddison & Maddison, 1992) and the consensus tree of the combined analysis of 18S rDNA and *rbc*L (Fig. 3). Within asterids, 768 unambiguous base changes were found, 77% of which are transitions (Table 1). Transitions involving cytosine and thymine occur twice as often as transitions between guanine and adenine, although the bias in base composition is

Table 2. Relative rates of change among stem bases of 18S rDNA at parsimony-informative sites in comparison with relative rates of change among all informative sites.

	A⇔C	A⇔G	A⇔T	C⇔G	C⇔T	G⇔T
Stem bases	0.10	0.49	0.07	0.10	1.00	0.22
All	0.28	0.67	0.23	0.19	1.00	0.30

not strong enough to explain this (A-19%, C-25%, G-32%, T-24%). The fact that thymine and cytosine are in more equal proportion than adenine and guanine may, however, partly explain the bias toward T↔C transitions, which seems to be consistent among all angiosperms (P. Soltis & D. Soltis, 1998), but not in animal rDNA, where both types of transitions occur at approximately the same rate (Vawter & Brown, 1993). This T↔C transition bias is even visible among all sites (Table 2), although it is not as pronounced as in stem bases alone, again in contrast to results from animal rDNA (Vawter & Brown, 1993), where the bias seems to be even stronger.

*rbc*L

The *rbc*L data set had 567 parsimony-informative sites, 531 of which were parsimony-informative within asterids. As in the 18S rDNA data set, the analysis did not run to completion and the analysis was again interrupted when 5000 trees of one length were found. The shortest trees found had 5909 steps (CI = 0.227; RI = 0.524). The strict consensus tree is shown in Figure 5.

COMBINED 18S/*rbc*L

This analysis included 178 taxa, 25 of them in the outgroup, with 3283 characters per taxon. The same positions that were excluded in the 18S rDNA analysis were omitted here. Of the 3177 characters used in the phylogenetic analysis, 851 were parsimony-informative, and 773 within asterids. In contrast to the analyses of the single data sets, the combined data set swapped to completion and yielded 80 shortest trees of 8374 steps (CI = 0.236; RI = 0.531). The strict consensus tree is shown in Figure 3.

*ndh*F

The *ndh*F data set contained 2370 aligned characters, of which 1377 were parsimony-informative and 1270 informative within the asterids. *ndh*F consists of two distinct regions. The 5′ portion extends to position 1340, just including the origin of replication (Ohtani et al., 1984) and lacked indels in the sequences sampled for this study (but see Olmstead & Reeves, 1995). In contrast, the 3′ portion of *ndh*F is characterized by frequent insertions and deletions. Consequently, the 5′ region has fewer parsimony-informative sites (608 versus 662), despite its greater length (1340 bp versus 1030 bp, respectively).

In the *ndh*F analysis, 21 shortest trees were found at 14,217 steps (CI = 0.239; RI = 0.480). The strict consensus tree is shown in Figure 6. Three taxa, *Hydrostachys*, *Galax*, and *Exacum*, take surprising positions in the analysis. *Hydrostachys* is, according to this analysis, together with *Galax* (Diapensiaceae) sister to *Eremosyne* in the euasterids II-clade. This position is likely due to problems in aligning the 3′ portion of *ndh*F. Contamination can be excluded based on a more in-depth analysis of the relationships of *Hydrostachys* including this *ndh*F sequence (Albach et al., in press a). The *ndh*F sequence of *Galax* is missing the first 614 bp. Therefore, *Galax* has not been included in an analysis of only the first region of *ndh*F. In trees of just one step longer than the most-parsimonious trees, *Galax* is sister to Styracaceae (incl. Halesiaceae), the position found in all of our other analyses. The position of *Exacum* as sister to the Solanaceae is harder to explain. Taxon sampling seems to be the decisive factor, because the same sequence showed gentianalean affinities with broader taxon sampling (Olmstead et al., 2000). Insertions and deletions in *ndh*F may be useful phylogenetic markers (e.g., Scotland et al., 1995). In the present study, 60 indels were encountered (Appendix 3) with twenty-six of them in only single taxa, but several other parsimony-informative.

*atp*B

The analysis of *atp*B sequence data included 1505 characters, 545 of which were parsimony-informative, with 514 of them informative within asterids. The analysis was stopped when 5000 trees of one length were reached. The shortest trees found were 4166 steps long (CI = 0.289; RI = 0.544). The strict consensus tree is shown in Figure 7.

COMBINED ANALYSIS OF FOUR GENES

The combined analysis of all taxa sequenced for all four genes included 7168 aligned characters, 3621 of them variable, and 2692 of them parsimony-informative with 2481 of them informative within the asterids. Analysis yielded eight most parsimonious trees of 25,199 steps (CI = 0.252; RI

Table 3. Mutations, transitions and transversions, and nucleotide frequency according to codon position in *rbc*L, *atp*B, and *ndh*F, measured on strict consensus tree of combined analysis of 18S rDNA, *rbc*L, *ndh*F, and *atp*B (see Fig. 2).

	*atp*B	*rbc*L	*ndh*F 5′	*ndh*F 3′	*ndh*F total
Unambiguous changes					
at first position	511 (14%)	699 (19%)	1172 (24%)	1684 (29%)	2856 (27%)
at second position	280 (8%)	412 (11%)	651 (13%)	1455 (25%)	2106 (20%)
at third position	2738 (76%)	2566 (70%)	3068 (63%)	2582 (45%)	5650 (53%)
T_i:T_v					
at first position	0.98	0.75	0.94	0.84	0.89
at second position	1.48	0.52	1.06	0.95	0.98
at third position	2.10	2.35	1.51	1.10	1.31
GC-content					
at first position	57.0%	58.4%	40.0%	33.3%	37.5%
at second position	41.8%	43.3%	38.6%	33.0%	36.4%
at third position	24.6%	30.5%	24.6%	22.9%	23.9%
Adenine					
at first position	27.0%	23.5%	29.3%	33.9%	31.1%
at second position	26.9%	29.8%	19.1%	30.8%	23.5%
at third position	34.3%	27.9%	28.5%	31.3%	29.6%
Cytosine					
at first position	18.2%	19.6%	15.6%	12.1%	14.3%
at second position	24.9%	22.5%	23.4%	18.3%	21.4%
at third position	14.0%	16.0%	9.2%	10.4%	9.6%
Guanine					
at first position	38.3%	38.8%	24.4%	21.2%	23.2%
at second position	17.0%	20.8%	15.2%	14.7%	15.0%
at third position	14.6%	14.5%	15.4%	12.5%	14.3%
Thymine					
at first position	16.0%	18.1%	30.7%	32.7%	31.5%
at second position	31.3%	26.8%	42.3%	36.2%	40.0%
at third position	37.1%	41.5%	46.9%	45.8%	46.5%

= 0.492). The strict consensus tree is shown in Figure 2.

Although the trees derived from separate and combined analyses are very similar, differences do exist. These differences may derive from different gene histories or from homoplasy in the separate data sets. Because differences due to different gene histories are less obvious on a level above the genus, the differences between the results of the separate and combined analyses are most probably due to homoplasy. The higher ratio for *atp*B compared with *rbc*L may be linked to the higher percentage of third position changes in *atp*B (Table 3).

The combined analysis of all four genes with 21 additional taxa that have been sequenced for only three genes found 2778 parsimony-informative characters and resulted in 24 most parsimonious trees at 27,568 steps (CI = 0.243; RI = 0.503). The strict consensus trees of the two four-gene analyses differ only in some relationships in Ericales (Figs. 1, 2A) and by showing greater resolution for Hydrangeaceae and Lamiales from the 137-taxon data set. Discussion is therefore centered on the larger 158-taxon data set.

Discussion

Molecular Evolution

Transition-transversion biases varied among the four genes. Transitions were equal to or more frequent than transversions, with T_i:T_v ratios ranging from 1.06 for *ndh*F to 1.98 for 18S rDNA (Table 4). A lower transition-transversion bias could indicate relaxed selection in a gene. In that context, it is noteworthy that the 5′ portion of *ndh*F has a considerably higher transition-transversion bias than the more variable 3′ region (1.36:1 versus 1.07:1; estimated for a most-parsimonious tree from the four-gene analysis reducing unidentified homoplasy in the analysis). GC content in the 5′ region is higher (34.2% versus 29.7% in 3′), approaching the mean of 38% for cpDNA (Zurawski & Clegg, 1993). Kim and Jansen (1995) also noted differences in variability, GC content, T_i:T_v ratios,

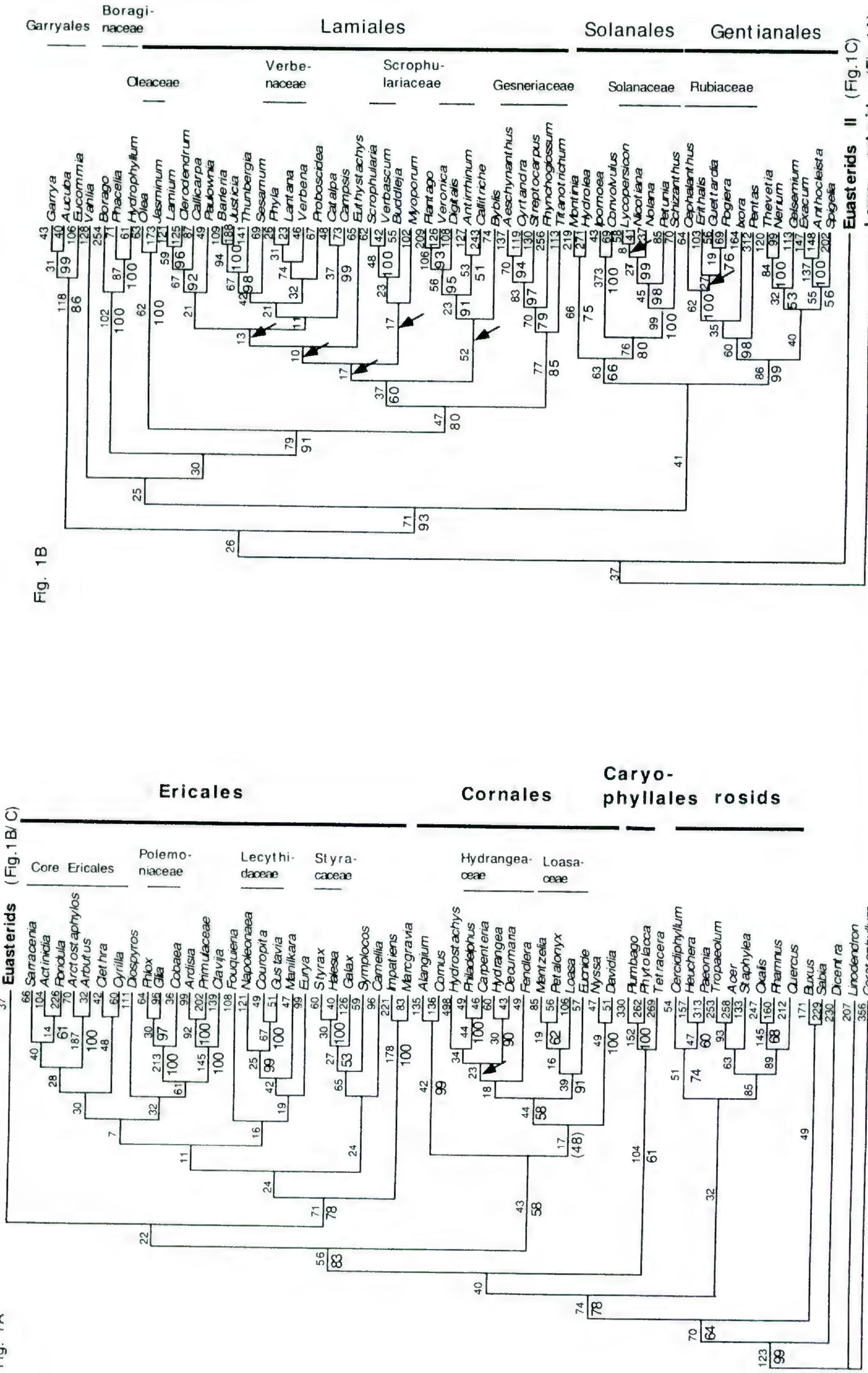


Figure 1 (pp. 170. 171). One of 24 most parsimonious trees from combined analysis of 18S rDNA, *rbc*L, *ndh*F, and *atp*B including taxa that have been sequenced for only three of these genes; numbers above the branches represent bootstrap values expressed as percentages of 500 replicates. Arrows indicate branches not present in the strict consensus tree. —A. Outgroup, Cornales, and Ericales. —B. Euasterid I. —C. Euasterid II.

Fig. 1C

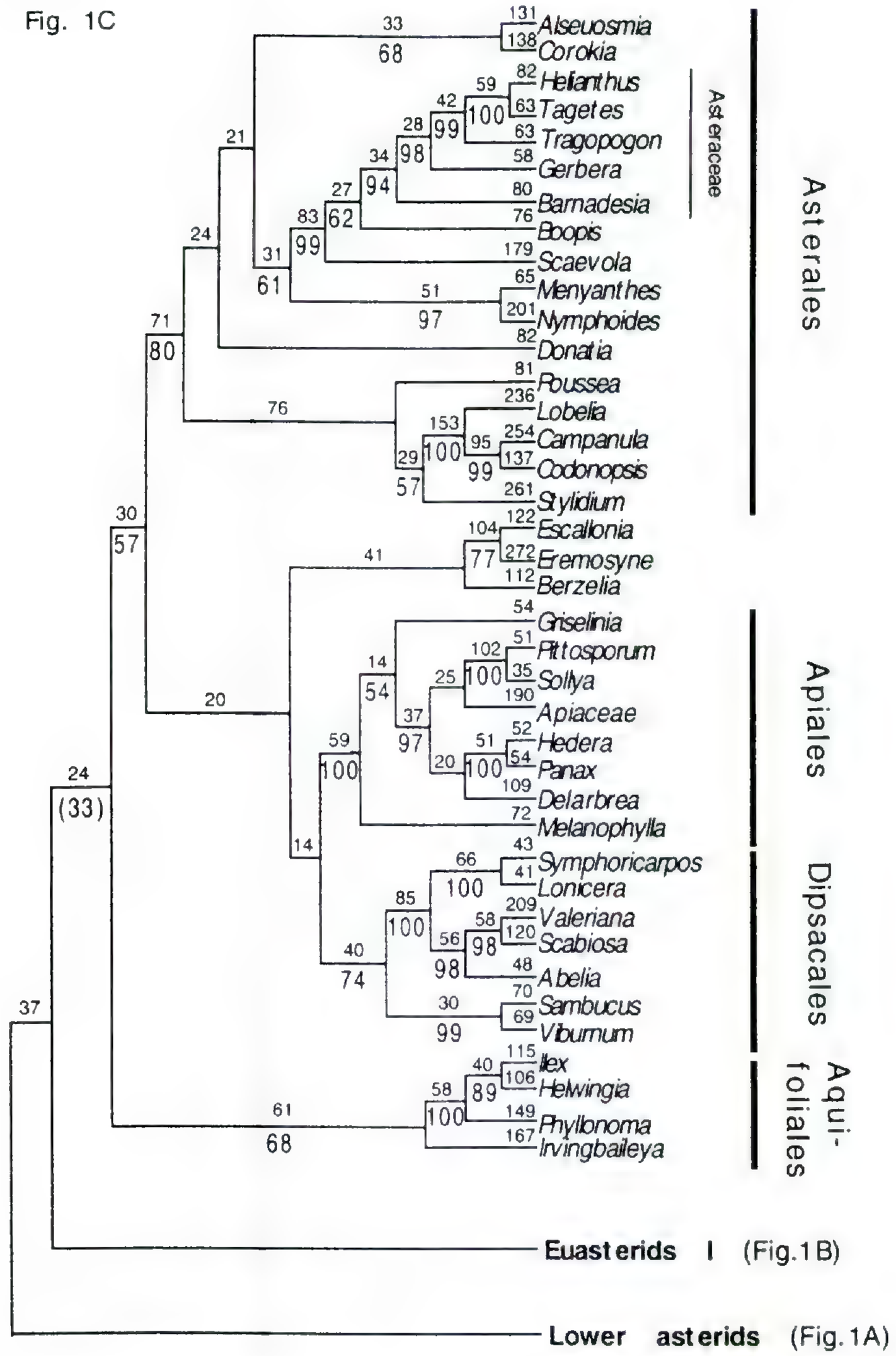


Figure 1. Continued.

and Ks/Ka values in the two regions of *ndh*F (divided at position 1380) in Asteraceae. They found the same pattern as herein, although the $T_i : T_v$ ratio is somewhat lower in their analysis (0.99 versus 1.06; Kim & Jansen, 1996: 10380 versus Table 3). The variability between the 5′ and 3′ regions is greater among the Asteraceae than among the asterids as a whole.

Base composition in the chloroplast genes is considerably biased toward adenine and thymine (Zurawski & Clegg, 1993; Tables 3, 4). However, *atp*B and *rbc*L are strikingly similar in base composition even among codon positions. In *ndh*F, the GC-content also decreases from first to third positions, but this decrease is less pronounced in the 5′ region of the gene than in *rbc*L and *atp*B, and is nearly undetectable in the 3′ region. The increased AT-content, increased number of unambiguous mutations per site, reduced transition bias, and especially the decreased differences of these characters among codon positions give further evidence for decreased selection pressure on *ndh*F. Because the 3′ portion shows this pattern even more strongly than the 5′ portion, selection may even be different between the two portions of *ndh*F analyzed. *ndh*F differs strikingly from both *atp*B and *rbc*L in amino acid composition (Table 5) with guanine-rich leucine, glycine, alanine, and valine the most common amino acids in *atp*B and *rbc*L and adenine- and thymine-rich amino acids serine, phenylalanine, and isoleucine being among the four most common amino acids in *ndh*F.

SYSTEMATIC IMPLICATIONS

The monophyly of asterids (Olmstead et al., 1992, 1993) has been confirmed by analyses of all

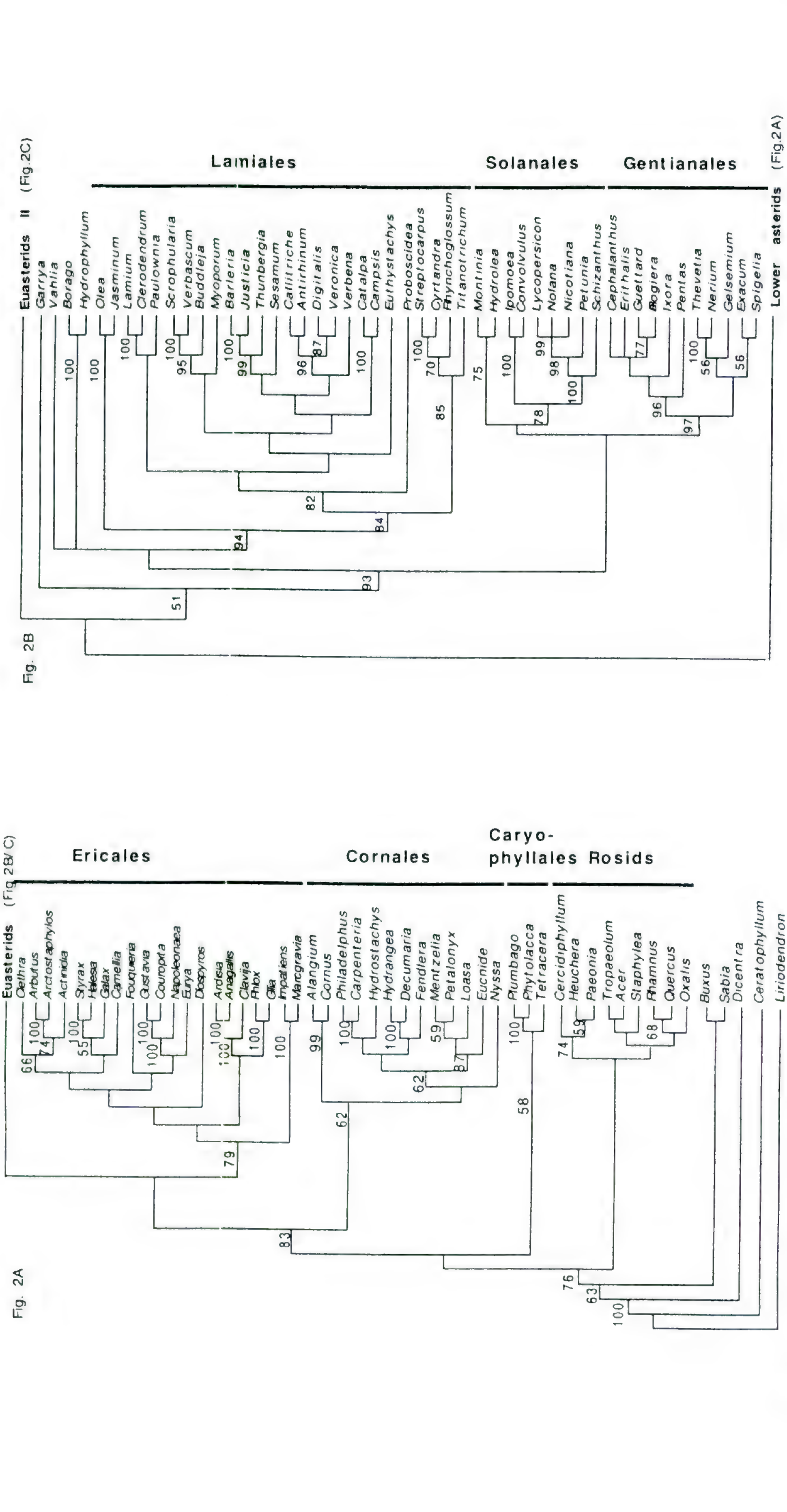


Figure 2 (pp. 172, 173). Strict consensus tree from combined analysis of 18S rDNA, *rbc*L, *ndh*F, and *atp*B; numbers above the branches represent bootstrap values expressed as percentages of 500 replicates. —A. Outgroup, Cornales, and Ericales. —B. Euasterid I. —C. Euasterid II.

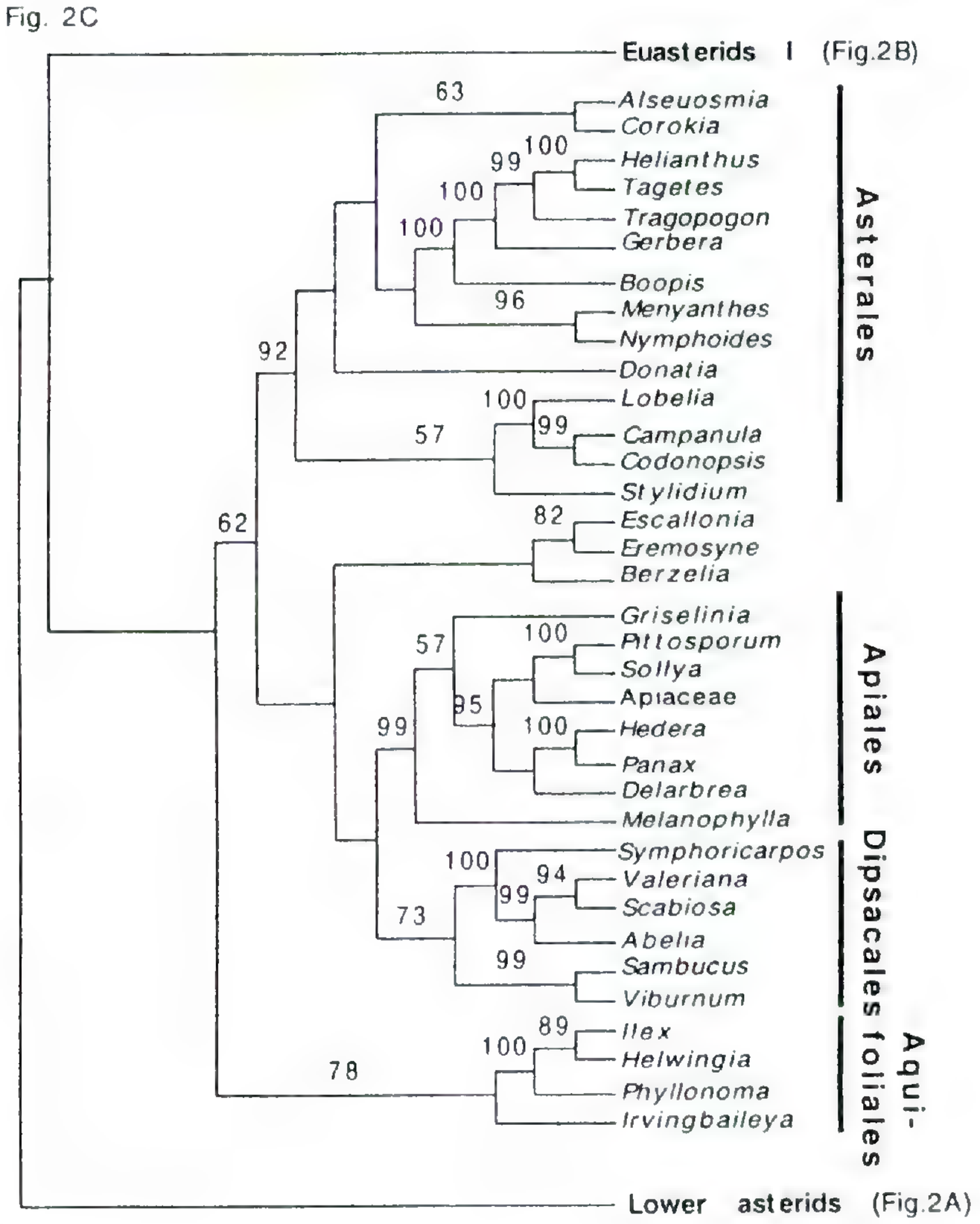


Figure 2. Continued.

four genes in both separate and combined analyses. In the combined analysis, the asterids are well supported (83% bootstrap, Fig. 1A). The Caryophyllales (sensu APG, 1998), plus Dilleniaceae, are sister to asterids based on the combined analysis of all four genes, as well as in the separate analyses of *rbc*L (Fig. 5A) and *atp*B (Fig. 7A). This is in agreement with an analysis of 18S rDNA, *rbc*L, and *atp*B sequence data for more than 500 species of angiosperms (Soltis et al., 2000). Analyses of *rbc*L (Olmstead et al., 1993) and a combined *rbc*L plus 18S rDNA data set (Fig. 3A) show the Caryophyllales nested within the rosids and this clade sister to the asterids. In the analysis of 18S rDNA presented here, however, Caryophyllales are sister to the rosid-asterid clade (Fig. 4A).

When choosing 155 of almost 80,000 species to represent asterids, most genera and even some fam-

Table 4. Characteristic values of the phylogenetic analyses. CI = Consistency index; RI = Retention index; underlined numbers—analyzed for the most parsimonious trees of combined four gene analysis (137 taxa); numbers in italics—137-taxa data set analyzed for most parsimonious tree of 178 taxa separate analysis; * only analyzed for 178 taxa data set.

	18S rDNA	*rbc*L	18S/*rbc*L	*ndh*F	*atp*B	Combined
taxa	178/137	178/137	178	137	137	137/158
# of characters	1749	1428	3187	2370	1505	7168
variable sites	449/416	815/761	1264	1709	735	3621
informative sites	284/255	567/514	851	1377	545	2692/2778
length of shortest tree	2321/*1865* 2012	5909/4662 4770	8374	14217/14459	4166/4312	25,199/27,568
# of shortest trees	>5000*	>5000*	80	21	>5000	8/24
CI	0.275/0.236	0.227/0.259	0.236	0.239	0.289	0.252/0.243
RI	0.593/0.580	0.524/0.508	0.531	0.480	0.544	0.492/0.503
GC-content	49.75%	44.2%	—	32.6%	42.8%	—
T_i/T_v	1.98/*1.98*	1.56/1.52	—	1.06/1.17	1.85/1.88	—

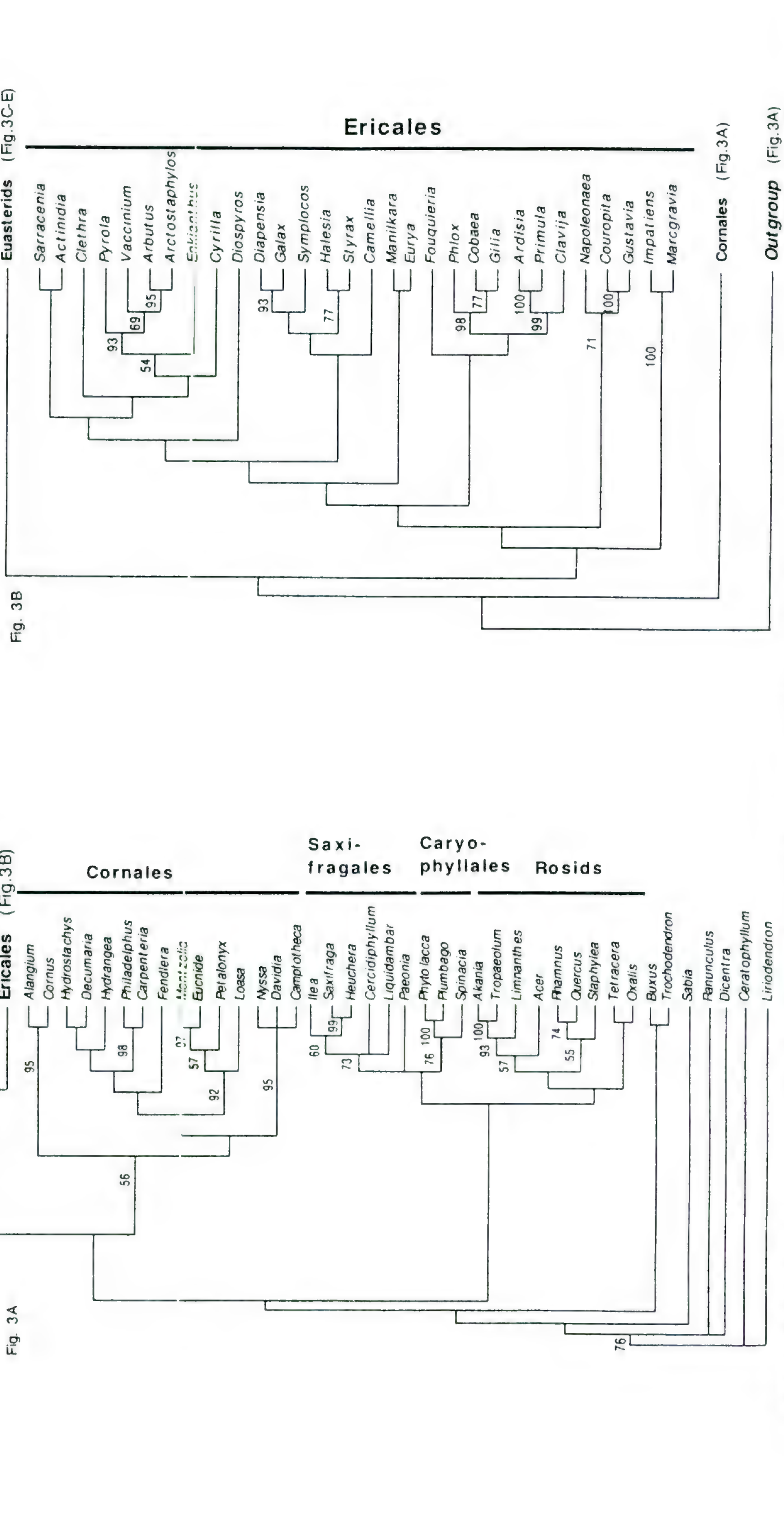


Figure 3 (pp. 174–176). Strict consensus tree from combined analysis of 18S rDNA and *rbcL*; numbers above the branches represent bootstrap percentages of 500 replicates. —A. Outgroup and Cornales. —B. Ericales. —C. Euasterid I. —D. Lamiales. —E. Euasterid II.

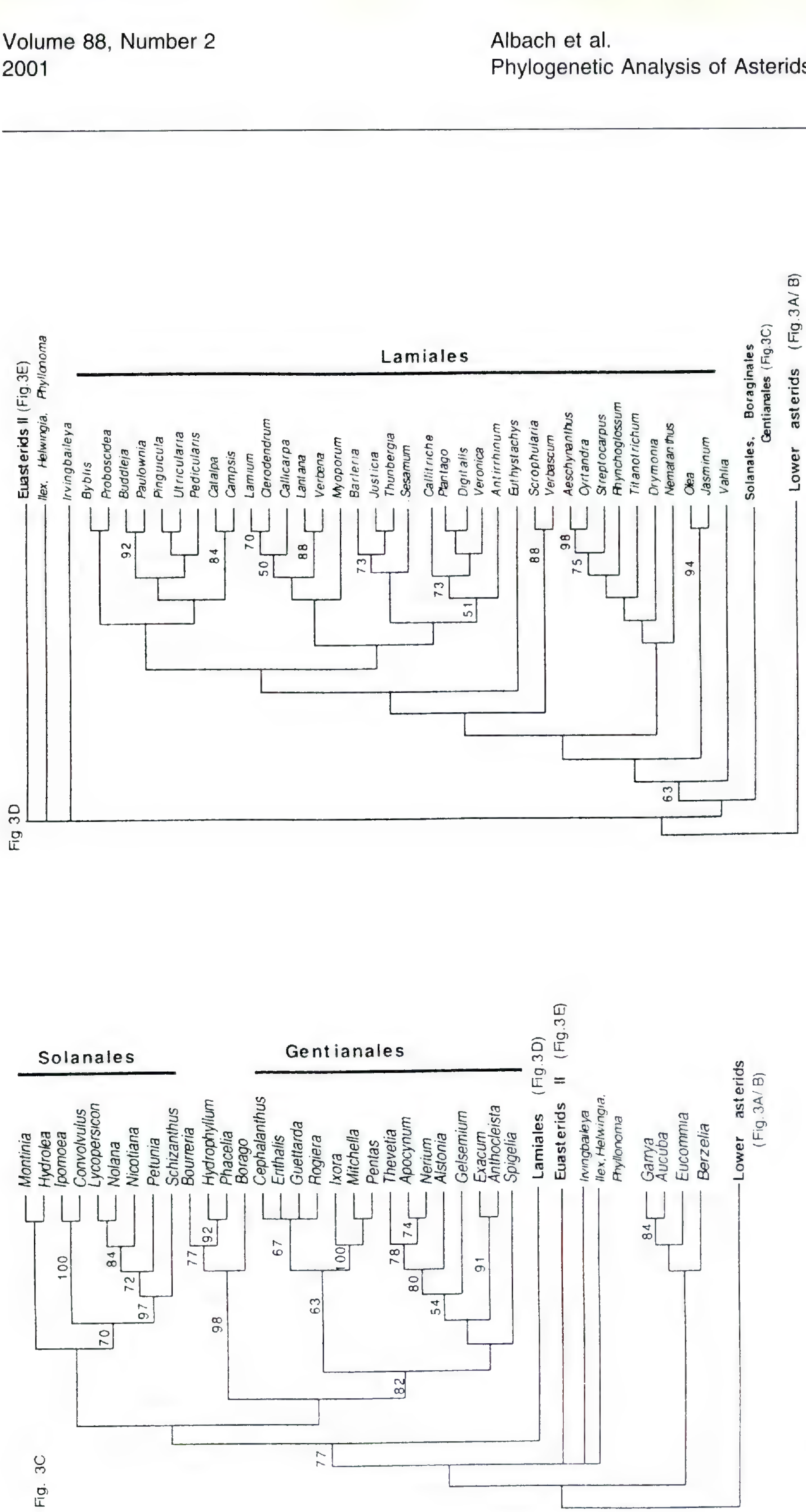


Figure 3. Continued.

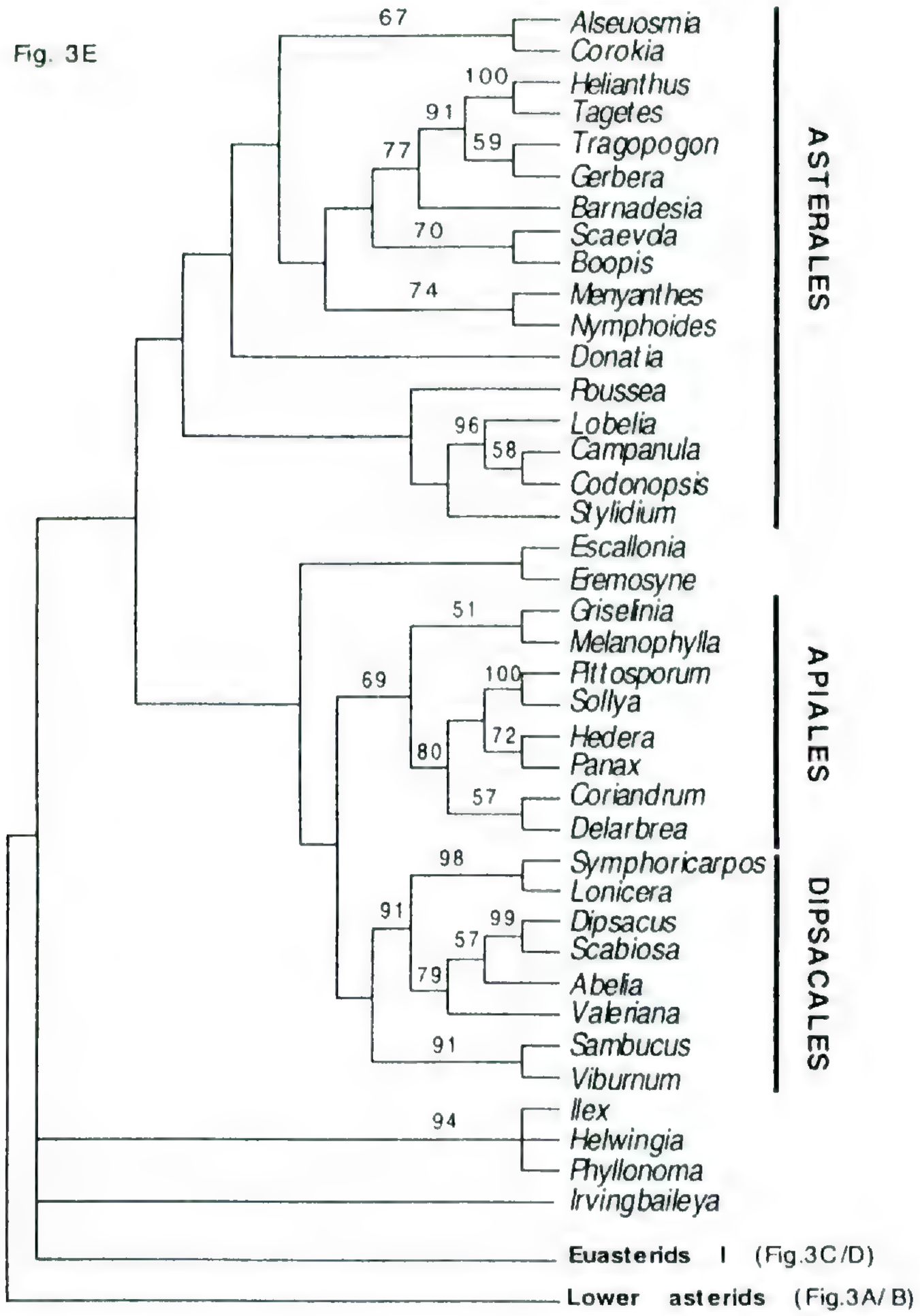


Figure 3. Continued.

ilies must be excluded. Some groups previously accepted as families have recently been shown to be derived from other families and have not been included here. Among these are Empetraceae, Epacridaceae, Monotropaceae (from Ericaceae; Anderberg, 1993; Kron, 1996), Scytopetalaceae (from Lecythidaceae; Morton et al., 1997), and Asclepiadaceae (from Apocynaceae; Sennblad & Bremer, 1996). Sampling of some orders was not extensive enough to resolve relationships within the order. This is especially true of the Lamiales where several critical families e.g., Avicenniaceae, Phrymataceae, Hippuridaceae, have not been included.

The asterids can be divided into four larger clades corresponding to asterid I–IV in Chase et al. (1993). The same clades, with no movement of taxa among them, were recovered in all analyses except for the separate 18S rDNA analysis. The Angiosperm Phylogeny Group (1998) called these clades euasterids I, euasterids II, Ericales, and Cornales. Cornales and Ericales, as well as two clades within both euasterid I (Garryales and the remainder) and euasterid II (Aquifoliales and remainder), have bootstrap values greater than 50%. Olmstead et al. (1993) recognized 11 different clades in the asterids. All clades have been found again and are supported by bootstrap values of more than 50%. *Escallonia* and *Berzelia* could not be positioned into one of these clades by Olmstead et al. (1993) or by the present analysis. The same is true for *Eremosyne* and *Vahlia* in the present analysis.

CORNALES

The Cornales (asterid IV sensu Chase et al., 1993; Cornales sensu Olmstead et al., 1993) consist of six families studied here: Cornaceae, Alangiaceae, Nyssaceae, Loasaceae, Hydrangeaceae, and Hydrostachyaceae (Fig. 1A). This conforms to the Cornales of Cronquist (1981), plus the inclusion of Hydrangeaceae (Rosidae–Rosales), Hydrostachyaceae (Asteridae–Callitrichales), and Loasaceae (Dilleniidae–Violales). Excluded are Garryaceae,

Table 5. Amino acid frequency in *rbc*L, *atp*B, and *ndh*F as a percentage of all amino acids; N = A, C, G, or T; Y = C or T; R = G or A.

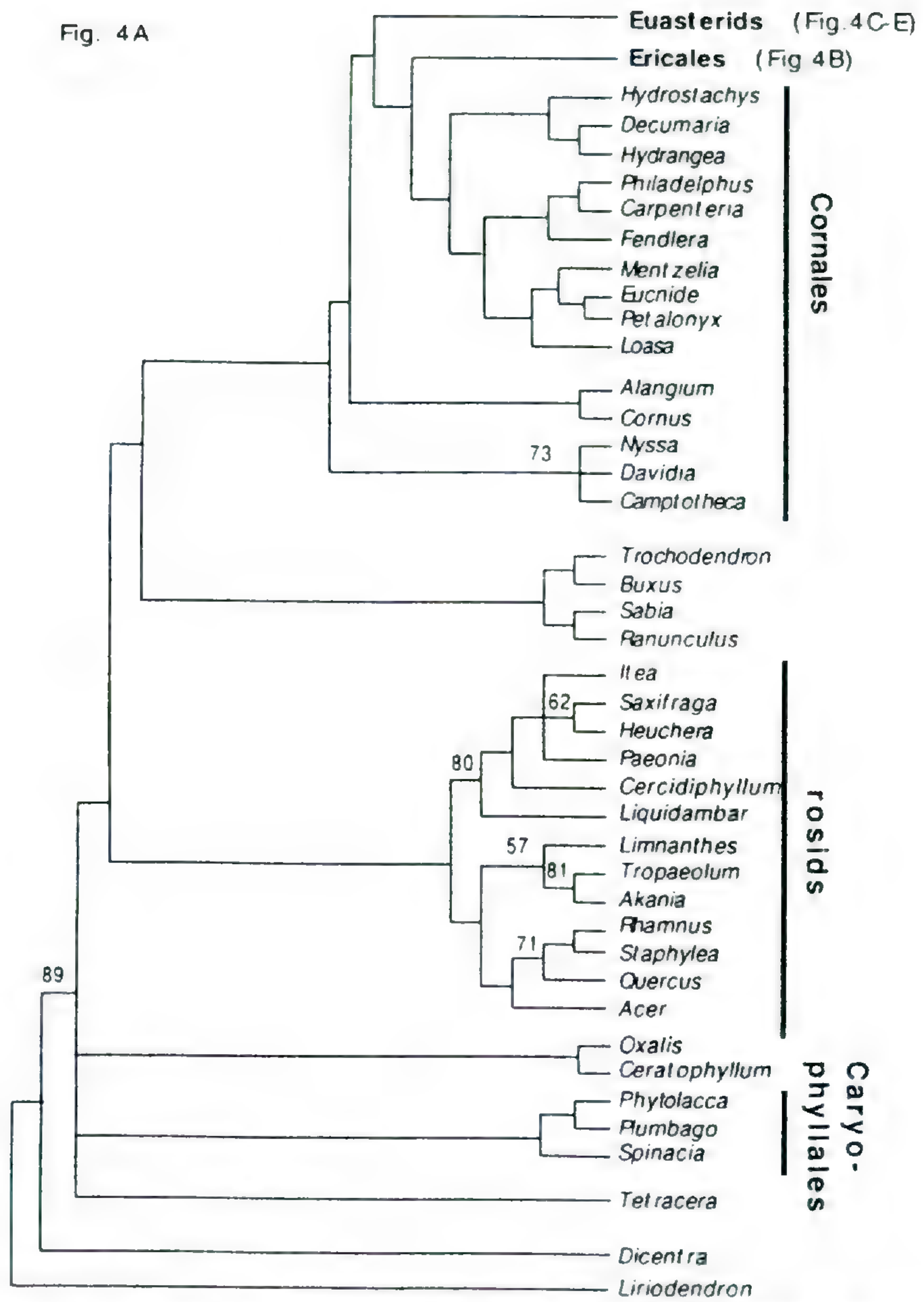
Amino acid	Codon	*rbc*L	*atp*B	*ndh*F 5′	*ndh*F 3′
Leucine	TTR/CTN	9.7	9.6	14.7	10.7
Glycine	GGN	8.6	9.9	6.8	6.7
Alanine	GCN	7.9	8.4	7.6	3.1
Valine	GTN	6.7	8.8	5.5	4.8
Arginine	CGN	6.6	5.7	2.3	4.1
Glutamic acid	GAR	6.0	6.7	1.9	2.7
Threonine	ACN	5.9	7.0	5.6	5.2
Lysine	AAR	5.0	3.7	2.5	6.1
Serine	TCR/AGY	4.9	5.3	8.5	9.4
Isoleucine	ATR	4.9	6.6	9.9	9.1
Aspartic acid	GAY	4.7	4.8	2.5	4.2
Proline	CCN	4.5	5.2	4.1	3.2
Phenylalanine	TTY	4.3	3.6	7.6	9.5
Tyrosine	TAY	3.9	2.5	4.3	6.1
Asparagine	AAY	3.1	3.9	3.6	7.5
Histidine	CAY	2.8	0.9	1.8	2.0
Glutamine	CAR	2.4	4.0	2.6	1.7
Cysteine	TGY	2.4	0.3	1.3	0.7
Methionine	ATG	2.1	2.8	4.3	1.4
Tryptophane	TGG	1.9	0.2	2.4	1.6

Aucubaceae, and Helwingiaceae, with the latter two related to Garryaceae and Aquifoliaceae, respectively. Aralidiaceae, Griseliniaceae, Melanophyllaceae, and Toricelliaceae were not included in this analysis, but belong to an expanded Apiales (Plunkett, 1994; Plunkett et al., 1997). Our Cornales resemble more closely the Cornales of Takhtajan (1997), although he did not include Hydrostachyaceae or Loasaceae in Cornales, but placed them in Lamiidae. Thorne's (1992) Cornineae resemble Cronquist's Cornales, but include Eucommiaceae and Icacinaceae, both of which show other relationships in this study. Members of Cronquist's Cornales and Thorne's Cornineae occupy early-diverging branches in three of the four major clades of asterids, sharing several plesiomorphic characters and forming a paraphyletic backbone of this clade (Fig. 1). Previous cladistic analyses using *rbc*L (Olmstead et al., 1992, 1993; Chase et al., 1993; Xiang et al., 1993), restriction sites (Downie & Palmer, 1992), and morphological and chemical data (including Garryaceae, Sarraceniaceae; Hufford, 1992) have revealed this same cornalean clade. In the circumscription used herein (including *Curtisia* and *Mastixia* not sampled here), the Cornales include 40 genera, and approximately 660 species.

The Cornaceae and Alangiaceae form a clade with 99% bootstrap support, and this clade is sister to the remaining Cornales. These two families resemble each other in floral morphology and embryology, but differ in biochemistry and some morphological features (Eyde, 1988). They share germination valves in their seeds, a base chromosome number of 11, and transseptal vascular bundles to the ovules with Nyssaceae (*Nyssa, Camptotheca, Davidia*), the next branch of the tree (Figs. 1A, 3A). Cornaceae and Nyssaceae (and *Mastixia*) also share the presence of ellagitannins (Bate-Smith et al., 1975), which separates them from genera such as *Aucuba, Corokia, Griselinia,* and *Helwingia* that have been included in Cornales by Cronquist (1981). In analyses based on *rbc*L alone, Xiang et al. (1993) and Soltis et al. (1995) found the reverse order of branching, with Nyssaceae the sister to the remaining Cornales. Hydrangeaceae–Loasaceae are sister to all other Cornales in a combined analysis of *rbc*L and *mat*K (Xiang et al., 1998).

Loasaceae and Hydrangeaceae appear as sister groups in all analyses (Figs. 1–7A), as they did with morphological and chemical data (Hufford, 1992) and *rbc*L alone (Hempel et al., 1995; Soltis et al., 1995). Although Loasaceae were assumed to be a member of the Asteridae, supported by the occurrence of iridoids and unitegmic-tenuinucellate ovules (Jensen et al., 1975), a relationship with Hydrangeaceae had not been proposed before Hufford's (1992) analysis. Both families have rare iridoids (deutzioside and derivates, C10-decarboxylated iridoids; El-Naggar & Beal, 1980) and share tuberculate trichomes with basal cell pedestals (Hufford, 1992). Some Hydrangeaceae (*Fendlera*) and Loasaceae show very similar vessel elements (Quibell, 1972). The relationship between Hydrangeaceae and Loasaceae is also supported by floral morphology (Roels et al., 1997), but these characters might be symplesiomorphies (L. Hufford, pers. comm.). The relationships within Hydrangeaceae are the same as those found previously using *rbc*L and broader taxon sampling (Soltis et al., 1995).

The position of Hydrostachyaceae, aquatic plants from Madagascar and eastern Africa, is a bit more complex. The sequences of *Hydrostachys* are highly divergent from those of Hydrangeaceae and Loasaceae, and sequences of *Hydrostachys* for 18S rDNA and *ndh*F show several unique insertions and deletions. All of the analyses show a relationship of *Hydrostachys* with Hydrangeaceae and Loasaceae (58% bootstrap support in the combined analysis of all four genes), but at varying positions (for *ndh*F, see Results). In the 18S rDNA/*rbc*L analysis, *Hydrostachys* appears with *Decumaria* within Hydrangeaceae (Fig. 3A; see also Hempel et al., 1995), but in the combined analysis of all four genes it is sister to *Philadelphus* and *Carpenteria* (Fig. 1A). However,



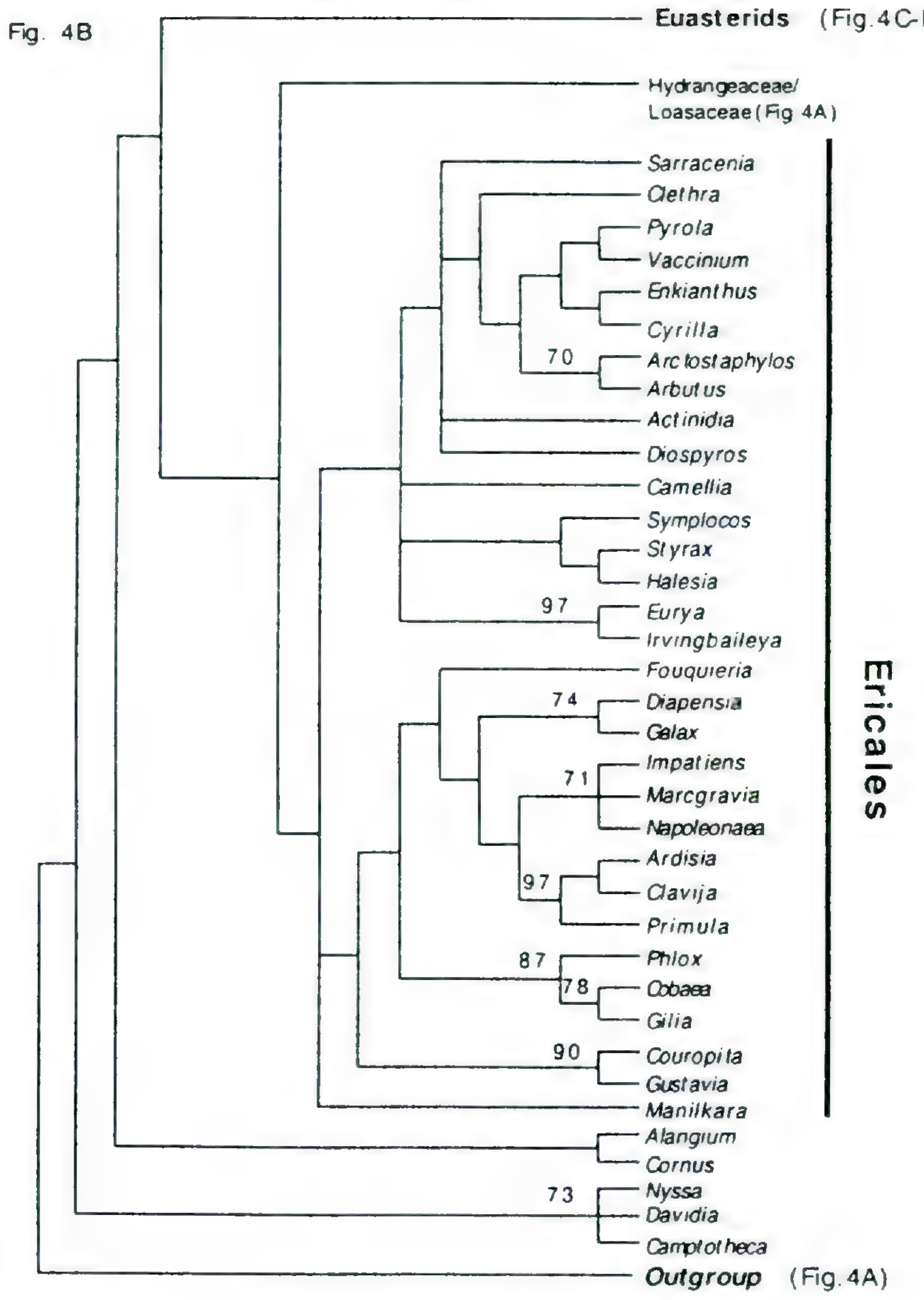


Figure 4 (pp. 178–180). Strict consensus tree from analysis of 18S rDNA; numbers above the branches represent bootstrap values expressed as percentages of 500 replicates. —A. Outgroup and Cornales. —B. Ericales. —C. Euasterid I. —D. Lamiales. —E. Euasterid II.

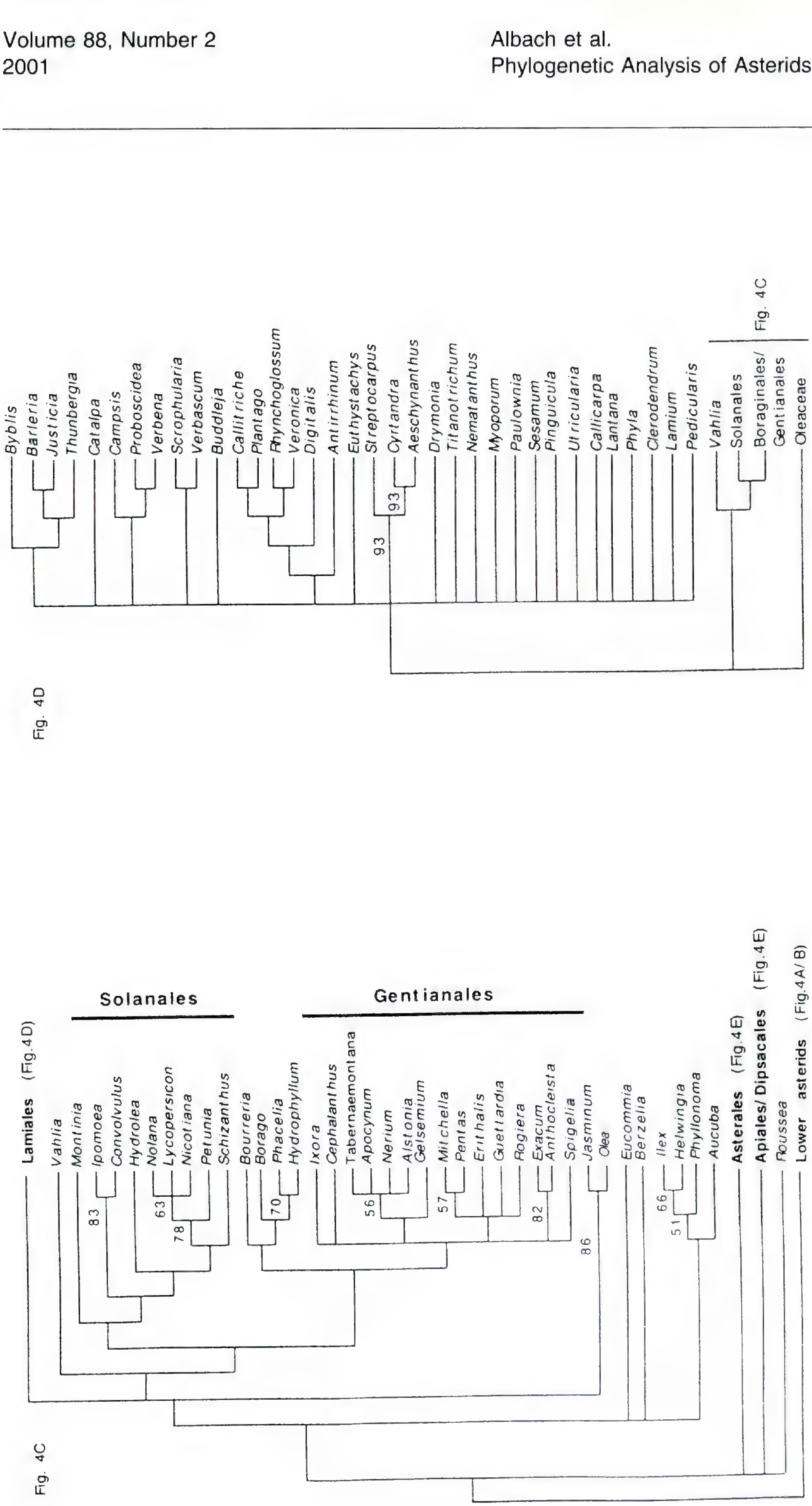


Figure 4. Continued.

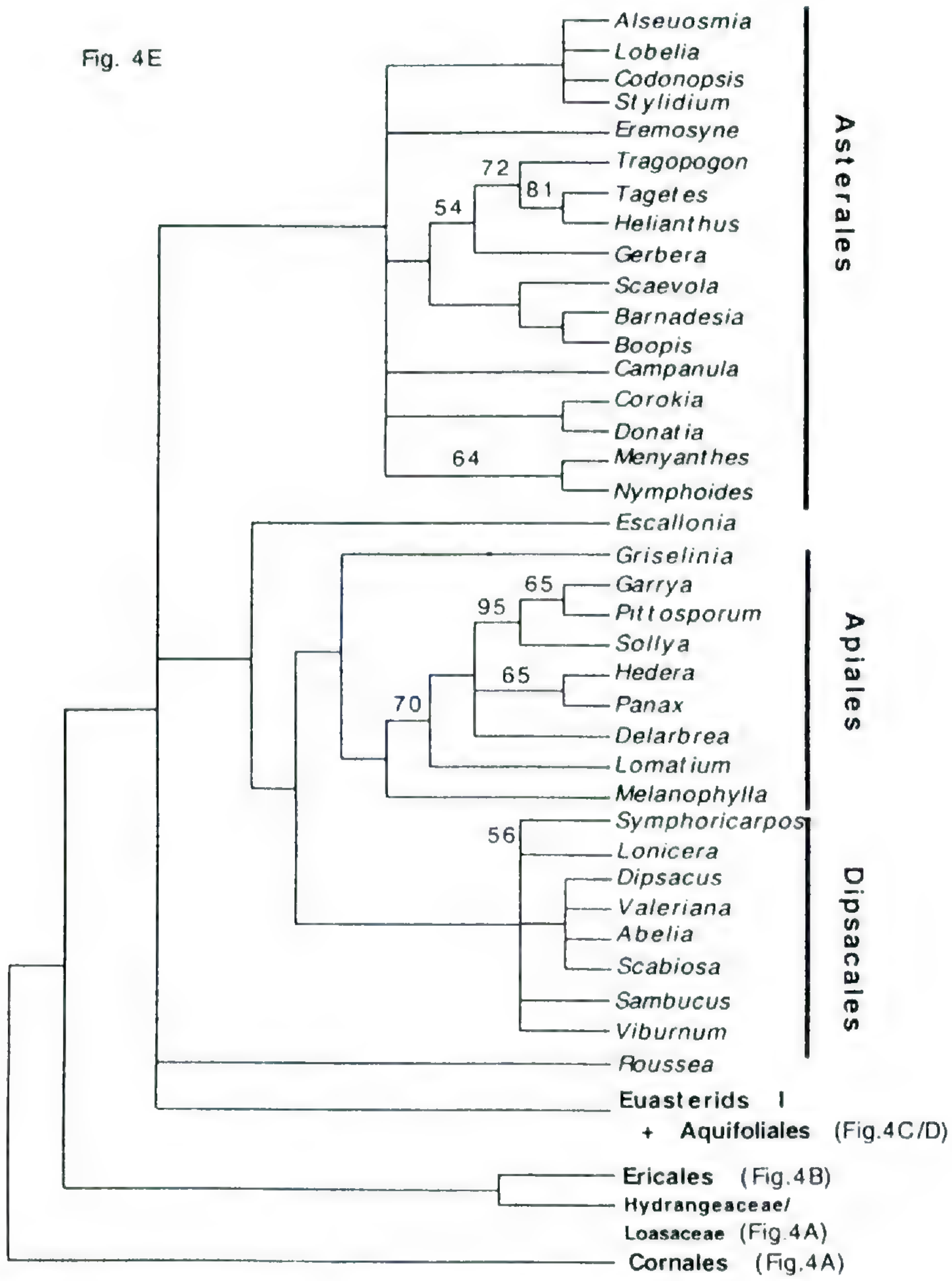


Figure 4. Continued.

an analysis of Hydrangeaceae with better taxon sampling is necessary to identify the closest relatives of *Hydrostachys*.

ERICALES

The Ericales (asterid II sensu Chase et al., 1993; Ericalean clade sensu Olmstead et al., 1993) consist mainly of taxa traditionally placed in Dilleniidae (sensu Cronquist, 1981), including many Theales, the Lecythidales, Ericales, Diapensiales, Ebenales, Primulales, Fouquieriaceae, and Sarraceniaceae, as well as Polemoniaceae (Asteridae) and Balsaminaceae (Rosidae) (Fig. 1A). Support is relatively high (78% bootstrap support, Fig. 1A) corroborating results by Källersjö et al. (1998; 85% jackknife support). Altogether at least 22 families with almost 10,000 species in about 350 genera are included. No study before Olmstead et al. (1992) had ever recognized such a clade. The Ericales differ from most of the other asterids by the common occurrence of ellagic acid, a compound frequently found outside the asterids but not within it. The relationships within this clade are still unclear. Ebenales sensu Cronquist (1981), for example, including Ebenaceae, Lissocarpaceae, Sapotaceae, Styracaceae, and Symplocaceae, share only such characters as woodiness, simple leaves, radially symmetrical flowers, axile placentation, tenuinucellate ovules, and a few large seeds (Morton et al., 1996), characters they also share with many families included here in Ericales, especially the Cyrillaceae, Clethraceae, "Primulales," Polemoniaceae, and Theaceae. Only a few well-supported clades appear within this large group. Of the seven groups of families identified by Morton et al. (1996), only three were recovered by our analyses: Ericaceae and allies, Primulales (sensu Cronquist, 1981), and Balsaminaceae/Marcgraviaceae. These taxa and the clade of Diapensiaceae and Styracaceae (incl. Halesiaceae) are the only clades of more than one family within the Ericales that have boot-

strap support of more than 50% (also Soltis et al., 2000). Even with the combination of four genes, the branches among these taxa are relatively short with further resolution to be sought.

Balsaminaceae and Marcgraviaceae form a clade that is sister to the rest of the Ericales. The analyses of Morton et al. (1996, 1997) reveal the same clade, plus *Tetramerista* (not sequenced here). In the analysis by Källersjö et al. (1998), *Pelliciera* was also added to this clade. This clade is a surprising combination, because a close relationship had never been proposed between these families. This seems improbable based on different habits with herbs in Balsaminaceae but climbing shrubs in Marcgraviaceae. They are, however, a highly supported clade with 100% bootstrap support. They share the presence of raphides and specialized nectaries or glands on leaves, petioles, sepals, or petals (Morton et al., 1996). Additionally, they share bitegmic-tenuinucellate ovules with cellular endosperm and endothelium, mostly simple perforation plates in vessels, and hypogynous flowers.

The "Primulales" (sensu Cronquist, 1981), consisting of Primulaceae, Myrsinaceae, and Theophrastaceae, are a highly supported clade (100% bootstrap support). The sister-group relationship of Theophrastaceae to Primulaceae and Myrsinaceae is supported not only by molecular data, but also by morphological data (Anderberg & Ståhl, 1995). The sister group to "Primulales" appears to be Polemoniaceae (*rbc*L, *ndh*F, and combined analysis; Figs. 5B, 6A, 1A, respectively), as found in Chase et al. (1993). In other analyses based on *rbc*L (Morton et al., 1996) and 18S rDNA (Johnson et al., 1999) that included "Primulales," Ebenaceae (Morton et al., 1996) or Balsaminaceae (Johnson et al., 1999) alternately appear as sister to "Primulales." Polemoniaceae are sister to *Halesia* and Diapensiaceae (Morton et al., 1996) or Fouquieriaceae (Johnson et al., 1999). Two characters that unite "Primulales" and Polemoniaceae are nuclear endosperm and simple vessel perforation plates. Neither character is restricted to these two taxa within Ericales, but they occur in combination elsewhere only in Sapotaceae. Another character that is shared by at least some "Primulales" and Polemoniaceae is the occurrence of separate sepals in *Cobaea*, *Polemonium*, and some genera of Theophrastaceae and Myrsinaceae, generally a rare condition among sympetalous plants (Stebbins, 1974). Additionally, Primulaceae and Polemoniaceae (but not *Cyclamen* and Myrsinaceae) both initiate stamen primordia earlier than petal primordia (Nishino, 1978, 1983). Biochemically, Polemoniaceae and Primulaceae resemble each other in many characters, such as cucurbitacins, and plants in both families excrete methylated 6- or 8-oxygenated flavonols from glandular hairs among many other compounds common to many Ericales (Hegnauer, 1990). Anderberg & Ståhl (1995) chose Sapotaceae and not Polemoniaceae as the outgroup for their morphological analysis of the Primulales for reasons not stated.

Hegnauer (1990) noted a biochemical resemblance between Sapotaceae and Myrsinaceae, based on the presence of saponins, polyphenolic compounds, triterpenes, and leucodelphinidin (the last also occurring in Ebenaceae). In none of our analyses, however, are the Sapotaceae close to the Primulales. The association of Fouquieriaceae with Polemoniaceae and Primulales in the combined 18S rDNA/*rbc*L analysis (Fig. 3B) is interesting, because a relationship between *Fouquieria* and *Acanthogilia*, a putatively little-derived lineage within Polemoniaceae (Johnson et al., 1996), has been suggested based on morphology (Nash, 1903; Henrickson, 1967, reviewed by Hufford, 1992), biochemistry (Scogin, 1977, 1978), and 18S rDNA sequence data (Johnson et al., 1999).

Diapensiaceae and Styracaceae (incl. Halesiaceae) form a clade in both the *rbc*L (Fig. 5B) and the four-gene combined analyses (Figs. 1A, 2A). In contrast to previous analyses based on *rbc*L (Morton et al., 1996, 1997) that showed *Halesia* and other genera of Styracaceae to be only distantly related to *Styrax*, the Styracaceae here always form a monophyletic group with 100% bootstrap support in the combined analysis (Figs. 1A, 2A), as found by Kron (1996) in a combined analysis of *rbc*L and 18S rDNA. However, several characters argue for a split within Styracaceae and a closer relationship of some Styracaceae with Diapensiaceae. Styracaceae are heterogeneous cytologically, with *Styrax* having $x = 8$ and *Halesia* $x = 12$; Diapensiaceae have $x = 6$, 9, 12 (Raven, 1975; Goldblatt, 1985). Embryologically, *Halesia* (and all other genera except for *Pamphilia*) differs from *Styrax* in having unitegmic ovules, as do Diapensiaceae (Dickison, 1993). *Halesia* and *Styrax* also differ in fruit type (capsule fruit versus stone fruit, respectively) and fatty acid composition (Breuer et al., 1987). Characters that unite *Halesia* and Diapensiaceae are given by Morton et al. (1996), but contain many plesiomorphies. Not mentioned by Morton et al. (1996) is the shared development of the embryo following the *Chenopodium* variation of the Polygonad type (Yamazaki, 1974). Several of these characters are also present in Symplocaceae, such as crystals of calcium oxalate, unitegmic ovules, unilacunar nodes, cellular endosperm, and binucleate pollen.

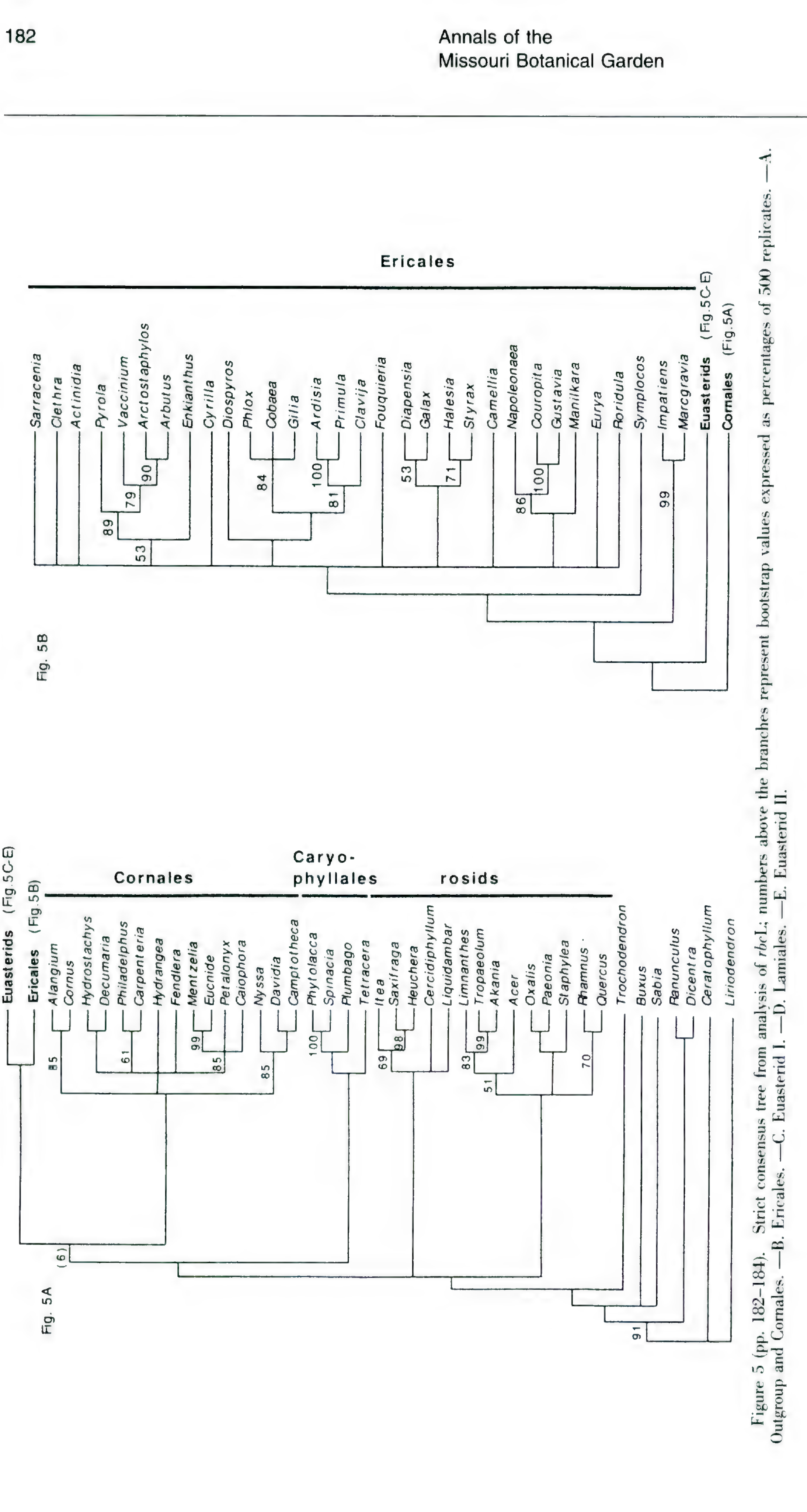


Figure 5 (pp. 182–184). Strict consensus tree from analysis of *rbc*L; numbers above the branches represent bootstrap values expressed as percentages of 500 replicates. —A. Outgroup and Cornales. —B. Ericales. —C. Euasterid I. —D. Lamiales. —E. Euasterid II.

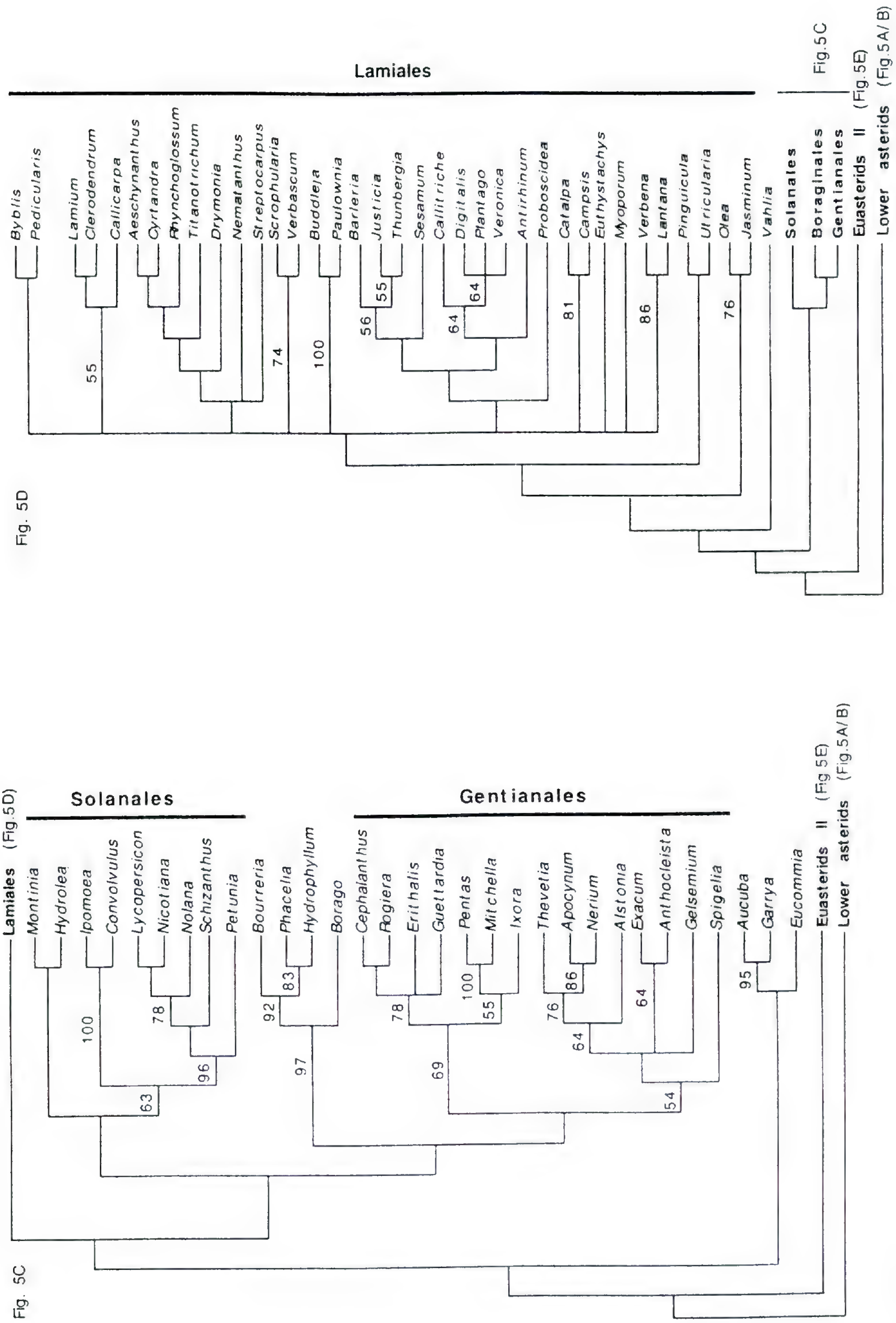


Figure 5. Continued.

Fig. 5E

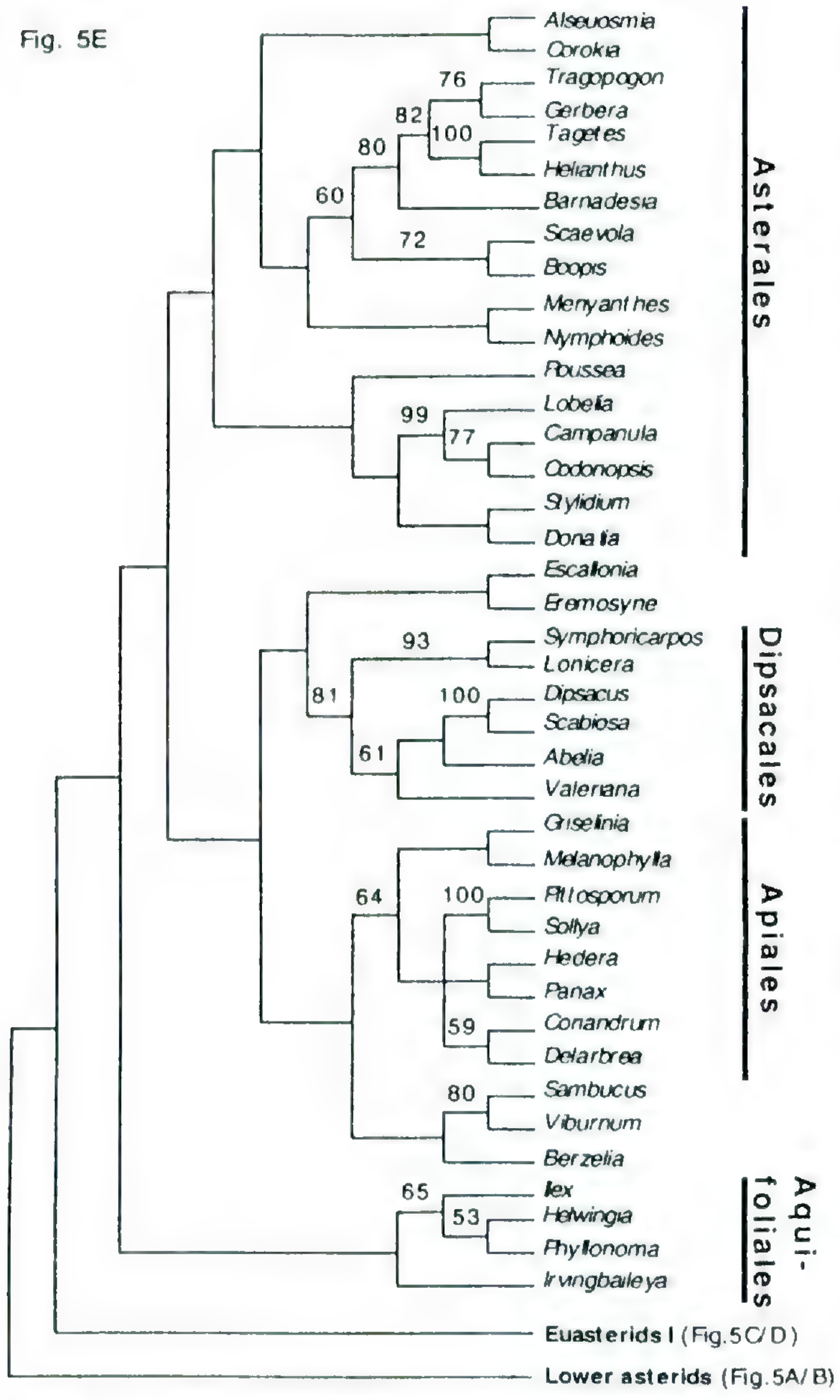


Figure 5. Continued.

The two former subfamilies of Theaceae (Theoideae, Ternstroemerioideae) do not form a clade in any of our analyses, confirming results by Morton et al. (1996) and supporting the recognition of two separate families, Theaceae and Ternstroemeriaceae (APG, 1998). These two families differ in many respects, such as embryological characters (Tsou, 1995), with characters used to unite the two subfamilies occurring among many Ericales. The positions of the two families are not clear. Theaceae, represented by *Camellia*, take a position at the base of Styracaceae and Diapensiaceae, as well as Sapotaceae in the 18S rDNA/*rbc*L analysis (Fig. 3B). In previous *rbc*L analyses (Morton et al., 1996, 1997), Theaceae were sister to Symplocaceae and Ericales (sensu Cronquist (1981) plus Actinidiaceae, *Roridula*, and Sarraceniaceae), respectively. Ternstroemeriaceae, represented by *Eurya*, are sister to Sapotaceae in the 18S rDNA/*rbc*L analysis (Fig. 3B) and to Lecythidaceae, when all four genes are combined (Fig. 1A). In the studies by Morton et al. (1996, 1997), Ternstroemeriaceae took alternative isolated positions as sister to different larger clades. Better taxon sampling and a faster-evolving gene are needed to evaluate the positions of Theaceae and Ternstroemeriaceae.

A clade consisting of Ericaceae, Actinidiaceae, Sarraceniaceae, Roridulaceae, Cyrillaceae, and Clethraceae occurs in the combined analyses, but this clade is not well supported (bootstrap value lower than 50%; Fig. 1A). Pyrolaceae (together with Monotropaceae, Epacridaceae, and Empetraceae) have been shown to be derived from within Ericaceae by morphological data (Anderberg, 1993), *rbc*L sequence data (Kron & Chase, 1993), and 18S rDNA sequence data (Kron, 1996). This clade of core Ericales plus Diapensiaceae has been found in analyses of morphological characters (Anderberg, 1993; Keller et al., 1996). *rbc*L analyses excluded either Clethraceae (Chase et al., 1993; Morton et al., 1996), Cyrillaceae (Chase et al., 1993), Sarraceniaceae (Morton et al., 1996), or Actinidiaceae (Kron & Chase, 1993) from this core Ericales clade. Core Ericales recovered in our analysis are characterized by inverted anthers (Anderberg, 1993), but other characters that are more heterogeneous are reviewed by Anderberg (1993). The group contains all of the iridoid-containing Ericales except for *Fouquieria* and *Symplocos*.

EUASTERIDS I

The euasterids I-clade (Asterid I sensu Chase et al., 1993) inferred from the analysis of all four genes (with 86% bootstrap support) consists of three larger clades and three smaller clades. The Garryales, consisting of *Eucommia*, *Garrya*, and *Aucuba*, are sister to the remaining euasterids I. The larger clades are Gentianales, Solanales, and Lamiales (sensu APG, 1998). *Vahlia* and Boraginaceae constitute smaller clades, possibly at the base of Lamiales (Fig. 1B).

The Garryales were also retrieved in earlier analyses using *rbc*L (Olmstead et al., 1993; Xiang et al., 1993). A relationship between *Garrya* and *Aucuba* is supported by quite a number of characters (e.g., palynological data; Ferguson, 1977) and was first proposed by Baillon (1879). Both *Garrya* and *Aucuba* are dioecious, evergreen shrubs or small trees with tetramerous flowers and opposite leaves. They both have petroselinic acid present in seed oils (Breuer et al., 1987), two-seeded fruits lacking a septum, and a similar gynoecial vasculature (Eyde, 1964) and several other characters. *Eucommia* shares, for example, a dioecious, woody habit with *Aucuba* and *Garrya*. All three taxa contain similar iridoid compounds: Aucubin has been de-

tected in all three genera (Jensen et al., 1975), while eucommioside has been found in *Eucommia* and *Aucuba* (Boros & Stermitz, 1991). *Eucommia* and *Aucuba* further share a pseudomonomerous ovary.

The position of *Vahlia* and Boraginaceae–Hydrophyllaceae among Gentianales, Solanales, and Lamiales is not completely resolved. The Boraginaceae have usually been placed in or close to the Solanales (Dahlgren et al., 1981; Thorne, 1992; Chase et al., 1993; Takhtajan, 1997). A position close to the Lamiales (four-gene analysis, Fig. 1B; *ndh*F, Fig. 6C) has been suggested by Cronquist (1981), who split Boraginaceae and Hydrophyllaceae and put Boraginaceae in Lamiales based on similarities between tropical, woody Boraginaceae and tropical, woody Verbenaceae. A position of Boraginaceae close to Verbenaceae is not supported herein. The only other studies to suggest a relationship between Boraginaceae and Lamiales are that of Olmstead et al. (1992), who found such a relationship in preliminary analyses, and the three-gene analysis of Soltis et al. (2000). Development of the embryo may provide a reason to group Boraginaceae with Oleaceae, where the Polygonad embryo type predominates, in contrast to Gentianales and Solanales, where the Solanad type occurs (Yamazaki, 1974). Boraginaceae also share terminal endosperm haustoria with Lamiales (except Oleaceae). A position of Boraginaceae as sister to the Gentianales, as suggested by the analysis of 18S rDNA (Fig. 3C) and *rbc*L (Fig. 5C), is supported by nuclear endosperm formation in both groups, in contrast to the cellular endosperm seen in Solanales and Lamiales.

Vahlia, a genus of five species from Africa and the Middle East, has usually been considered close to Saxifragaceae, alternately included there (Cronquist, 1981), or as a separate family (Dahlgren et al., 1981; Thorne, 1992; Takhtajan, 1997). Based on *rbc*L sequence data, Morgan and Soltis (1993) were the first to suggest that *Vahlia* occupies a position at the base of Lamiales, the position it takes in the analyses of *rbc*L (Fig. 5D), *ndh*F (Fig. 6B), as well as the four-gene analysis (Fig. 1B). *Vahlia* differs markedly from Saxifragaceae in having iridoids (Al-Shammary, 1991) and tenuinucellate, pendulous ovules. *Vahlia* also differs from all other taxa in the euasterids I by having bitegmic ovules.

The Lamiales, with almost 18,000 species in about 1100 genera, are taxonomically one of the most difficult clades in the asterids. They include the formerly separated Scrophulariales (e.g., Cronquist, 1981; Takhtajan, 1997). A distinction between Lamiales and Scrophulariales would at best render the Scrophulariales, as commonly described, paraphyletic with respect to Lamiales. The Lamiales, as herein, share several characters found in almost all taxa studied that are extremely rare outside of this clade: verbascoside; anthraquinones derived from the shikimic acid pathway; C11-decarboxylated iridoids (Jensen, 1992); hairs with a stalk of one or more uniseriate cells and a head of two or more vertical cells; young anthers with "placentoid" tissue; and fewer than five fertile stamens (Rahn, 1996).

The Oleaceae are well supported as a member of the Lamiales in all analyses (91% bootstrap support for the Lamiales with Oleaceae, 80% for Lamiales when deleting Oleaceae from the data set in the combined analysis of all four genes), although they were often put in the vicinity of Gentianales (e.g., Dahlgren et al., 1981). Oleaceae separate from the remaining Lamiales by their embryogeny, which is of the Polygonad type, in contrast to the Onagrad type in the remainder of the order. Gesneriaceae are the next clade in all analyses, distinct from most other Lamiales in their lack of iridoid compounds. The iridoids of higher Lamiales are characteristic in being decarboxylated at C11, a feature otherwise only present in Garryales and Ericaceae (Jensen, 1991).

Within the remaining Lamiales, only a few clades can be identified, because short branches cause major differences in topology when adding or deleting taxa. Scrophulariaceae s. str. plus *Buddleja* constitute one clade characterized by acylated rhamnosyl iridoids (Jensen et al., 1998). This clade, with the addition of *Selago,* corresponds to "scroph I" of Olmstead and Reeves (1995). As in their analysis and Oxelman et al. (1999), the combined analysis of all four genes reveals Myoporaceae as sister to this clade. Another clade ("scroph II" of Olmstead & Reeves, 1995) consists of some Scrophulariaceae (*Veronica, Digitalis, Antirrhinum*), Plantaginaceae, and Callitrichaceae. Olmstead and Reeves (1995) and Oxelman et al. (1999) demonstrated with increased taxon sampling that Hippuridaceae are also included in this clade. This scroph II clade is the only clade of more than three taxa in Lamiales with a bootstrap value greater than 90% (Fig. 1B). A third clade recognizable in all analyses is the combination of Acanthaceae and *Sesamum* (Pedaliaceae), also shown by Oxelman et al. (1999). Both taxa contain amyloid, an oligosaccharide not found in other Lamiales (Hegnauer & Hegnauer, 1992), as well as a common pollen morphology (Erdtman, 1952).

Lamiaceae and Verbenaceae appear as a common clade in the 18S rDNA/*rbc*L analysis (Fig. 3D).

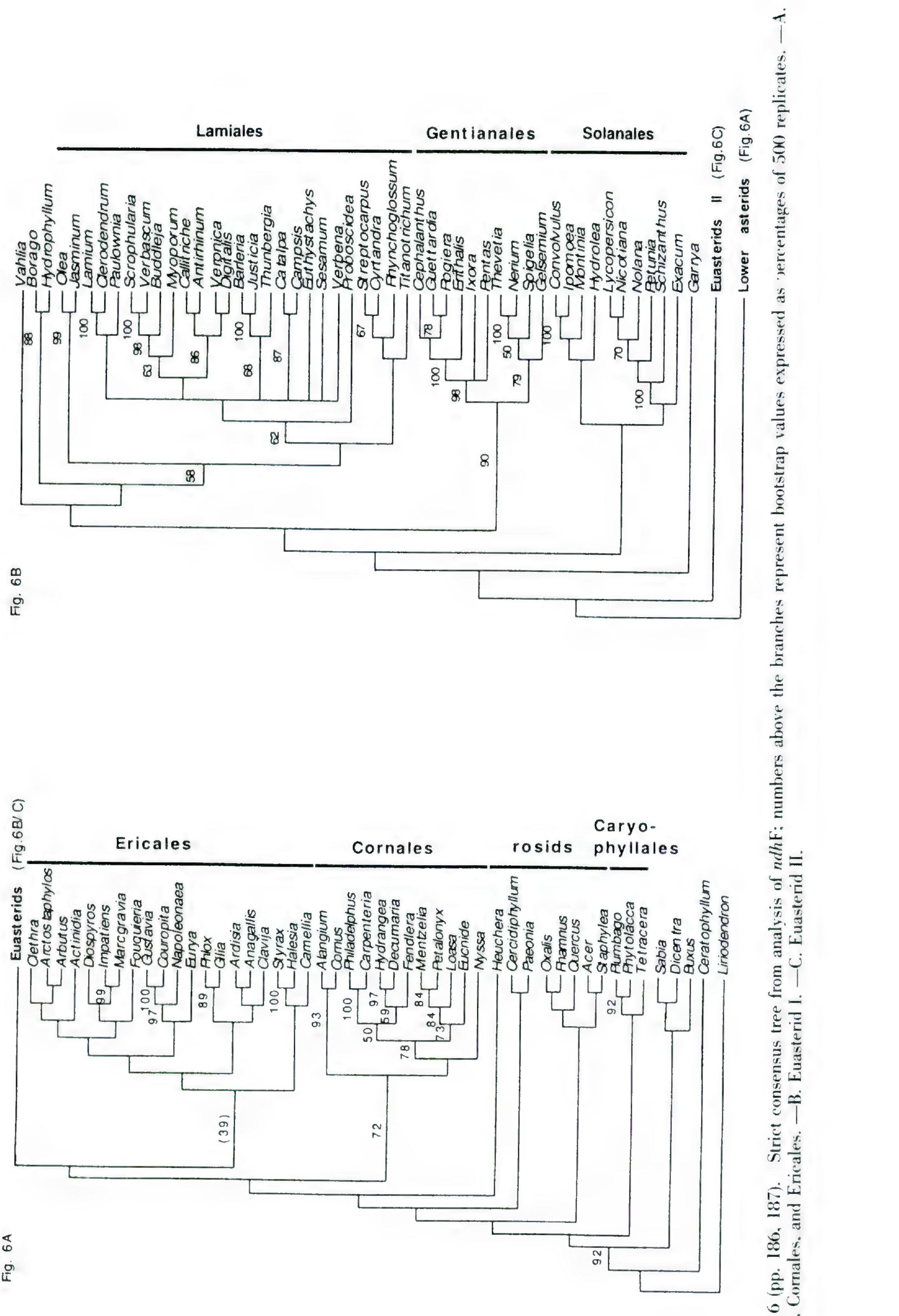


Figure 6 (pp. 186, 187). Strict consensus tree from analysis of *ndh*F; numbers above the branches represent bootstrap values expressed as percentages of 500 replicates. —A. Outgroup, Cornales, and Ericales. —B. Euasterid I. —C. Euasterid II.

Fig. 6C

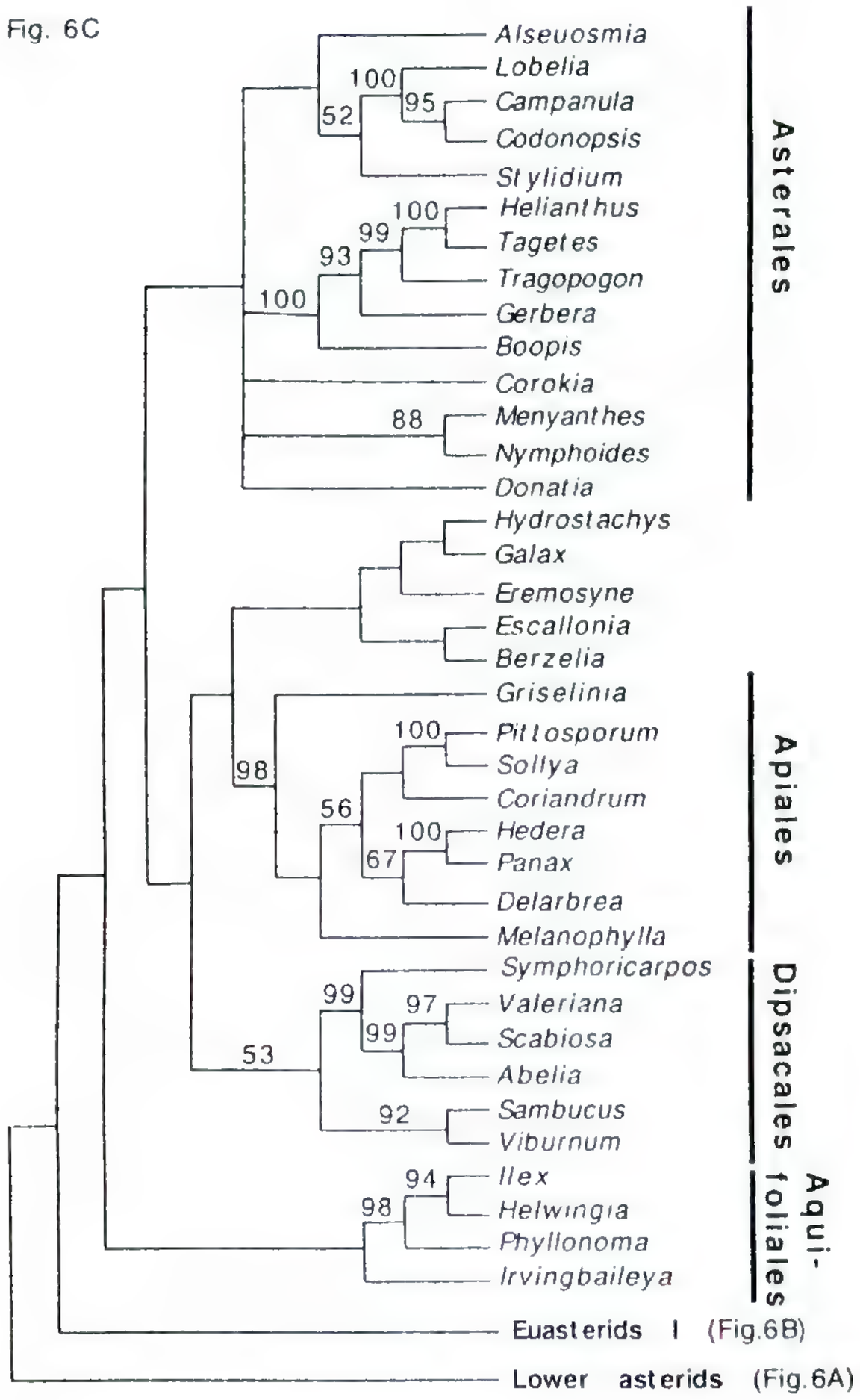


Figure 6. Continued.

In contrast, the combined analysis of all four genes (Fig. 1B) is congruent with the results of other studies using *rbc*L and *ndh*F (Olmstead & Reeves, 1995; Wagstaff & Olmstead, 1997; Wagstaff et al., 1998) in showing Verbenaceae s. str. and Lamiaceae s.l. (Cantino, 1992) in two distantly related clades. This placement further supports the recommendation of Cantino (1992) to restrict Verbenaceae to subfamily Verbenoideae, a clade that may not even be closely related to an expanded Lamiaceae. Verbenoideae differ from Lamiaceae and the other subfamilies of Verbenaceae in accumulation of 4-carboxy iridoids in the first versus C4-decarboxylated iridoids in the latter (von Poser et al., 1997).

The 18S rDNA/*rbc*L analysis groups *Byblis* and *Proboscidea* (Martyniceae), two carnivorous flypaper trap taxa (Albert et al., 1992). *Byblis* was generally believed to be related to Pittosporaceae (e.g., Cronquist, 1981; Dahlgren et al., 1981), while the Martyniaceae were put in the vicinity of Pedaliaceae and Bignoniaceae, and sometimes even included in Pedaliaceae (Cronquist, 1981). The relatively close relationship of *Byblis*, *Proboscidea*, and Lentibulariaceae suggests a common ancestry of the carnivorous syndrome with considerable subsequent structural divergence in this part of the Lamiales.

The Gentianales constitute a well-supported clade, including Rubiaceae, Apocynaceae, Gentianaceae, and Loganiaceae. The clade comprises approximately 1000 genera and 14,000 species. The Rubiaceae are sister to the remainder of the clade in all analyses except *atp*B (Figs. 1, 2, 6B, 3–5C). A common wood anatomy further supports this sister-group position of Rubiaceae. All Gentianales except Rubiaceae are characterized by intraxylary phloem (Carlquist, 1992). Previous *rbc*L studies (Chase et al., 1993; Olmstead et al., 1993; Bremer et al., 1994) and those for cpDNA restriction sites (Downie & Palmer, 1992) suggest this sister-group position for Rubiaceae as well. A morphological analysis of Struwe et al. (1994) differs, with Rubiaceae being sister to Gelsemiaceae and

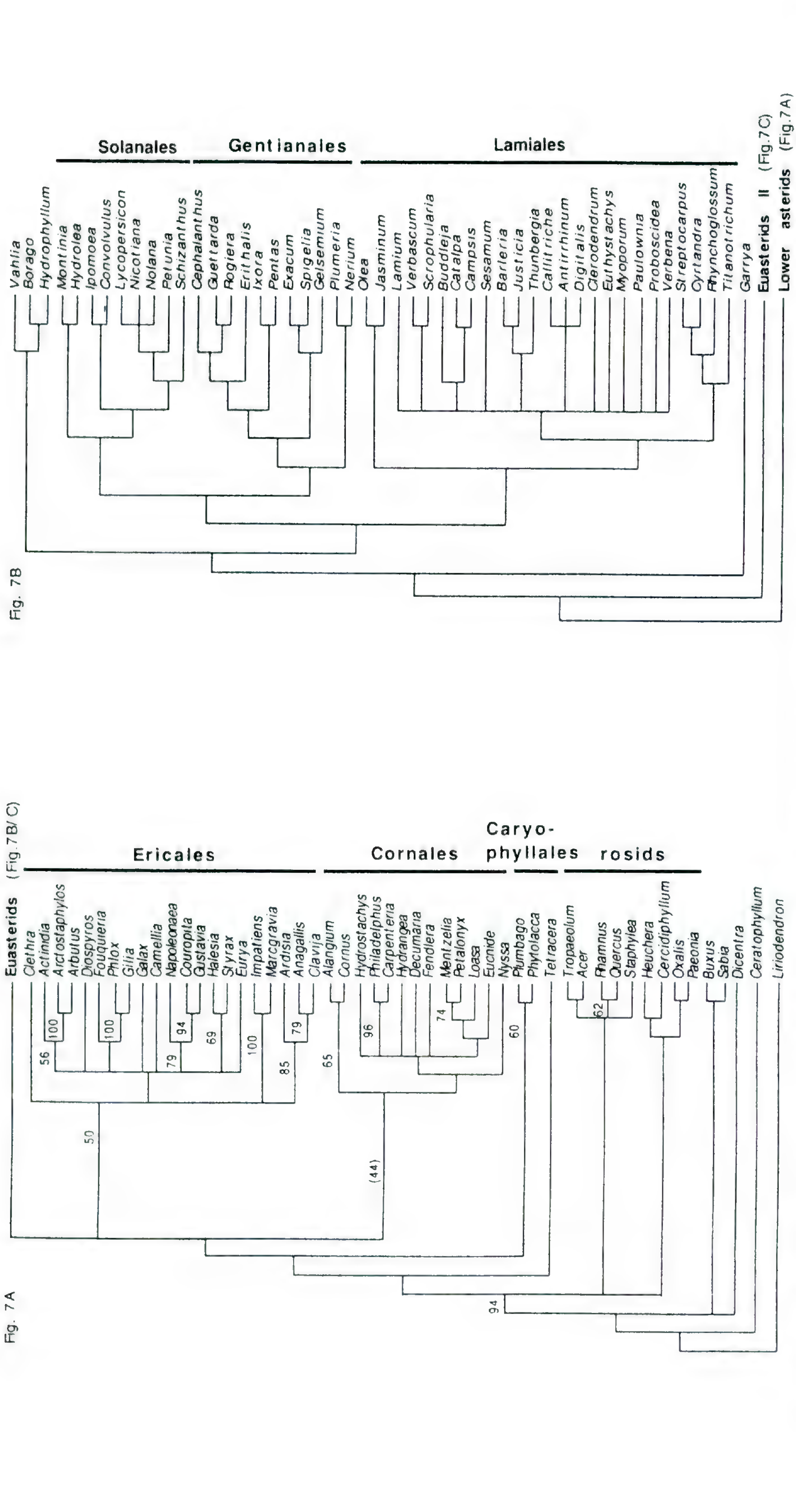


Figure 7 (pp. 188, 189). Strict consensus tree from analysis of *atp*B; numbers above the branches represent bootstrap values expressed as percentages of 500 replicates. —A. Outgroup, Cornales, and Ericales. —B. Euasterid I. —C. Euasterid II.

Fig. 7C

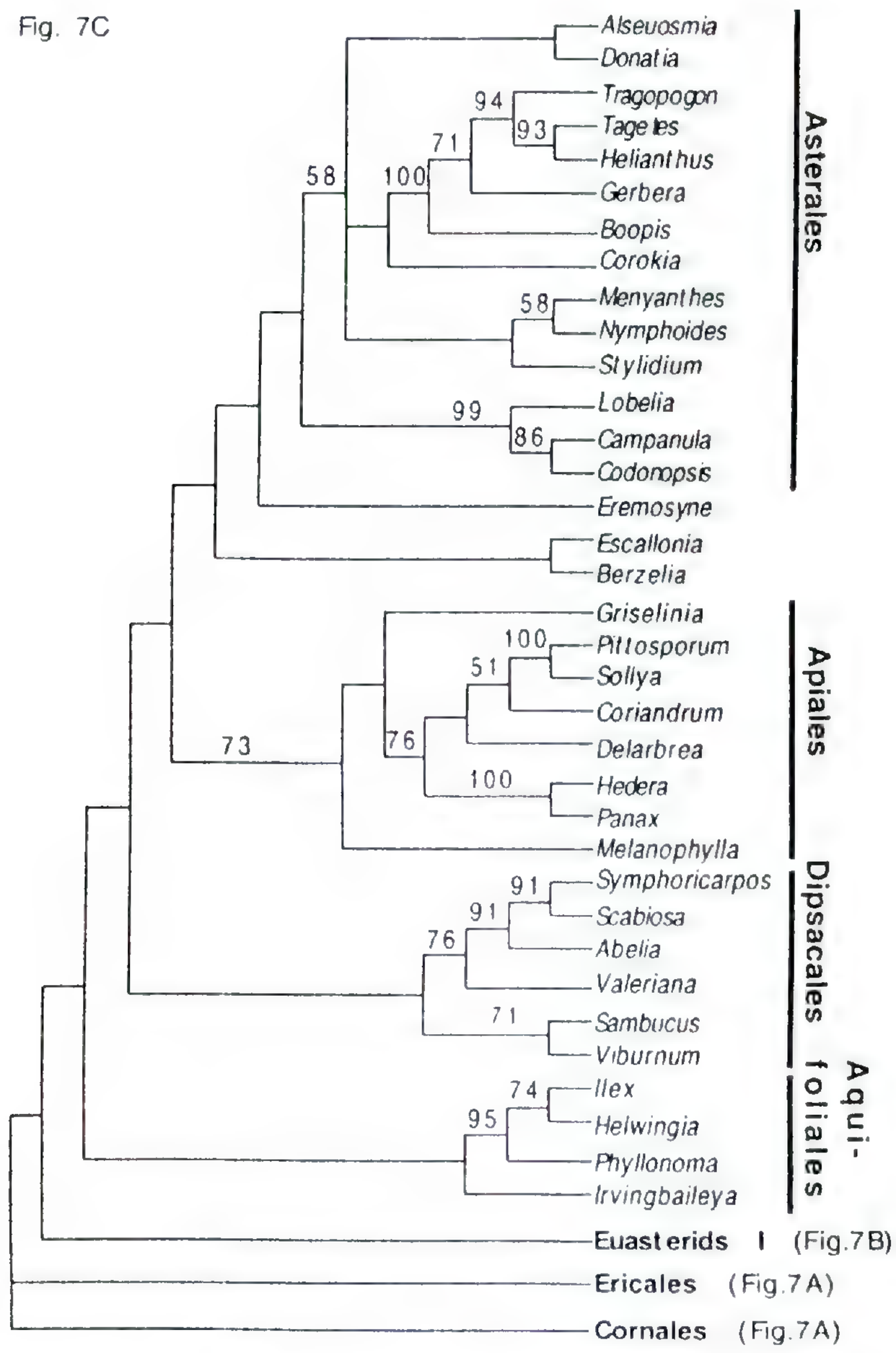


Figure 7. Continued.

the Loganiaceae s. str. at the base of the Gentianales. Taxon sampling in Gentianales, especially among Loganiaceae, is too limited in our study to resolve Gentianales. Polyphyly or paraphyly of Loganiaceae has been shown repeatedly (Figs. 1, 2, 6, 7B, 3–5C; Olmstead et al., 1993; Chase et al., 1993; Struwe et al., 1994; Bremer et al., 1994).

The Solanales, as circumscribed here (Fig. 1B, 66% bootstrap support), include Solanaceae, Convolvulaceae, *Montinia* (Grossulariaceae sensu Cronquist, 1981), and *Hydrolea* (Hydrophyllaceae sensu Cronquist, 1981). The Solanales comprise about 140 genera and 4800 species. Relationships within Solanaceae are congruent with results from previous analyses using *rbc*L, *ndh*F, and restriction sites (Olmstead & Sweere, 1994). Solanaceae and Convolvulaceae are chemically distinct from other euasterids I through the replacement of iridoids by tropane alkaloids (Jensen et al., 1975; Romeike, 1978). The split of Hydrophyllaceae, with *Hydrolea* assigned to Convolvulaceae and *Hydrophyllum* and *Phacelia* to Boraginaceae, was first proposed by de Jussieu (1789), but was later dismissed (Gray, 1875). Characters separating *Hydrolea* from other Hydrophyllaceae are bilocular capsule and axile placentation.

EUASTERIDS II

The euasterids II (asterid II sensu Chase et al., 1993) comprise five subclades (Fig. 1C). Three subclades correspond quite well to taxa recognized by Cronquist (1981) and Thorne (1992): Dipsacales, Asterales (Asteranae sensu Thorne), and Apiales. The fourth clade consists of *Escallonia* (Grossulariaceae sensu Cronquist; Hydrangeales sensu Thorne) and *Eremosyne* (Saxifragaceae sensu Cronquist; Saxifragales sensu Thorne). *Berzelia* is sister to *Escallonia* and *Eremosyne* (Fig. 1C). Sister to all of these euasterids II is Aquifoliales (bootstrap value of 68%), consisting of four taxa placed in Rosidae by Cronquist (1981): Aquifoliaceae, Icacinaceae (both Celastrales sensu Cronquist; Theales and Cornales sensu Thorne, not a member of Aquifoliales sensu APG, 1998), Helwingiaceae (Cornaceae sensu Cronquist; Araliales sensu Thorne), and

Phyllonomaceae (Grossulariaceae sensu Cronquist; Escalloniaceae sensu Thorne) (Fig. 1C). The remainder of the euasterids II form a clade that is supported by a 57% bootstrap value.

The euasterids II correspond to a combination of Asteridae and Cornidae–Aralianae sensu Takhtajan (1997) plus *Escallonia*, *Eremosyne*, and Icacinales (sensu Takhtajan, 1997). Icacinaceae may only partly belong to euasterids II as suggested by Savolainen et al. (2000), Soltis et al. (2000), and Albach et al. (in press a). The taxa of euasterids II are placed in Dahlgren et al.'s (1981) Araliiflorae (excluding Byblidaceae), Asteriflorae, Corniflorae–Dipsacales, and parts of his Corniflorae–Cornales. The Escalloniaceae are polyphyletic, with their members having different relationships within euasterids II. Other genera that seem to belong in this clade are *Columellia*, *Desfontainia*, *Phelline* (Backlund & Bremer, 1997), and *Sphenostemon* (V. Savolainen, A. Backlund & M. Chase, pers. comm.).

The euasterids II have also been supported in *rbc*L analyses (Chase et al., 1993; Olmstead et al., 1993; Cosner et al., 1994; Backlund & Bremer, 1997) and are supported by floral ontogeny (Roels & Smets, 1996) and mostly alternate leaves (with exceptions mainly in Dipsacales). This clade comprises many taxa characterized by secoiridoids or the apparent loss of iridoid production (the latter in Campanulaceae, Aquifoliaceae, and all Apiales except *Griselinia* and *Melanophylla*). Carbocyclic iridoids have been found only in five genera of euasterids II, three related genera of Icacinaceae that are also close to *Irvingbaileya* (Kaplan et al., 1991), *Stylidium* (Stylidiaceae), and *Escallonia* (Plouvier & Fabre-Bonvin, 1971). Iridoids and polyacetylenes seem to be complementary in distribution among euasterids II; they compete for acetyl-CoA and mevalonic acid as biosynthetic precursors (Stuhlfauth et al., 1985).

The first-branching clade of euasterids II, the Aquifoliales (*Ilex* clade sensu Olmstead et al., 1993), is represented by *Ilex*, *Irvingbaileya*, *Helwingia*, and *Phyllonoma*. In the analysis by Backlund and Bremer (1997), the Aquifoliales are sister to the Asterales rather than sister to all other euasterids II, as found here. The association of *Helwingia* and *Phyllonoma* is interesting, because they share the rare character of flowers borne on the leaf blades (Dickinson & Sattler, 1974). In the narrow circumscription of Aquifoliaceae, Icacinaceae (p.p.), Helwingiaceae, and Phyllonomaceae, this clade includes approximately 60 genera and 700 species. This circumscription assumes that Icacinaceae are monophyletic, which may not be the case (Savolainen et al., 2000; Soltis et al., 2000; Albach et al., in press a). The Icacinaceae still await more in-depth study with better taxon sampling.

The Asterales clade includes Asteraceae, Calyceraceae, Goodeniaceae, Menyanthaceae, Alseuosmiaceae, Argophyllaceae (*Corokia*), Donatiaceae, Campanulaceae, Rousseaceae, and Stylidiaceae (Fig. 1C). Additional families of Asterales include Carpodetaceae, Phellinaceae, and Pentaphragmataceae (Backlund & Bremer, 1997; Gustafsson & Bremer, 1997; Karehed et al., 1999) not sampled in our analysis. This circumscription differs from that of Lammers (1992) in the addition of Stylidiaceae and Donatiaceae. Lammers (1992) did not include Stylidiaceae because of the presence of carbocyclic iridoids in this family but not in the other families of this order. The production of carbocyclic iridoids may be the ancestral state based on the occurrence of these compounds in Cornales and early-diverging members of euasterids II. *Alseuosmia* and *Corokia* were not considered by Lammers (1992). Besides differences in taxon sampling, the relationships within this clade are with a single exception (see below) exactly the same as those found by Cosner et al. (1994) and are well supported (Fig. 1C). They are also congruent with the results of Gustafsson et al. (1996), although the latter study had slightly different taxon sampling. Backlund and Bremer (1997), Gustafsson and Bremer (1997), and Karehed et al. (1999) used broader taxon sampling (but not *Roussea*) and only *rbc*L sequences. Their result is, nevertheless, similar except for the position of *Donatia* (plus Abrophyllaceae), *Alseuosmia* (Alseuosmiaceae), and *Corokia* (Argophyllaceae plus Phellinaceae) relative to a clade of Asteraceae, Calyceraceae, Goodeniaceae, and Menyanthaceae. *Roussea* was first found to belong to Asterales by Soltis et al. (1997a) and more recently confirmed by Koontz and Soltis (1999). The Asterales in our circumscription include approximately 1250 genera and 23,000 species, dominated by Asteraceae. A common feature of Asterales is the presence of inulin (in Asteraceae, Calyceraceae, Campanulaceae, Goodeniaceae, Stylidiaceae). The basal split within this clade seems to be characterized by a difference in base chromosome number, with $x = 9$ in Asteraceae, many Goodeniaceae, Menyanthaceae, and *Corokia*, $x = 7$ in Campanulaceae, and $x = 15$ in Stylidiaceae. A possible synapomorphy for Campanulaceae and Stylidiaceae is the filamentous suspensor of the proembryo (Tobe & Morin, 1996).

Petal venation (Gustafsson, 1995) and loss of micropylar endosperm haustoria (Cosner et al., 1994)

are synapomorphies for the clade consisting of Asteraceae, Calyceraceae, Goodeniaceae, and Menyanthaceae, with *Corokia* and *Alseuosmia* (not investigated for haustorium) as sister to these four families. Other characters shared by these four families are multinucleate tapetal cells and the production of secoiridoids (Lammers, 1992). The production of secoiridoids, however, is plesiomorphic (Albach et al., in press b). In Asteraceae, the production of secoiridoids is mostly replaced by the production of sesquiterpene lactones (but see Changzeng & Dequan, 1997). The evaluation of synapomorphies within this order is, however, restricted by the lack of information on some of the lesser known families in this order.

One of the most debated issues in the evolution of Asterales is the sister group of the Asteraceae. Calyceraceae appear as the immediate sister with a 62% bootstrap value in the combined analysis of all four genes (Fig. 1C), as they were in an *rbc*L analysis by Gustafsson and Bremer (1997), while Calyceraceae and Goodeniaceae together are sister to Asteraceae in the 18S rDNA/*rbc*L analysis (Fig. 3E), as they were in previous *rbc*L analyses (Cosner et al., 1994; Gustafsson et al., 1996). Characters that support the sister-group relationship of Calyceraceae and Asteraceae are pollen morphology (Skvarla et al., 1977), petal venation, and morphology of the receptacles and receptacle bracts (Hansen, 1992). Biochemically, a relationship of Goodeniaceae and Calyceraceae is supported: both contain bis-secoiridoids of the sylvestroside type (Jensen et al., 1979; Capasso et al., 1996).

Escallonia and *Eremosyne* form a clade near the base of Apiales and Dipsacales in the four-gene analysis (Fig. 1C), but were more closely related to Dipsacales in analyses based on *rbc*L alone (Hibsch-Jetter et al., 1997; Fig. 5E) and to Apiales in the 18S rDNA analysis of Soltis and Soltis (1997; but see Fig. 4E). Trichomes with radially arranged apical cells in the glandular head may provide a synapomorphy for *Escallonia* and *Eremosyne* (Alshammary & Gornall, 1994).

Dipsacales as circumscribed here (Fig. 1C; 74% bootstrap support) correspond to Dipsacales sensu Cronquist (1981) and Thorne (1992) and Dipsacanae sensu Takhtajan (1997). They consist of Dipsacaceae, Valerianaceae, Caprifoliaceae, and Adoxaceae (including *Sambucus* and *Viburnum*), with approximately 400 genera and 1400 species. This circumscription differs from APG (1998) in the inclusion of Adoxaceae. Differences with other classifications are the inclusion of Calyceraceae (Dahlgren et al., 1981) and the placement of *Sambucus*, *Viburnum*, *Triplostegia*, and *Morina* in monotypic families (e.g., Takhtajan, 1997). The latter two were not included in our analyses but were shown to be members of Dipsacales by Backlund and Bremer (1997). Our sampling is insufficient to address familial status of *Sambucus* and *Viburnum*. Dipsacales in the circumscription herein are characterized by opposite leaves without stipules, an inferior ovary, a strong tendency toward zygomorphy and reduction in the number of stamens and carpels (Wagenitz, 1992), and trinucleate pollen. Rarely seen outside the Dipsacales (in Goodeniaceae, Calyceraceae, Oleaceae, and Icacinaceae) is the common presence of bis-iridoids (Jensen et al., 1979). Synapomorphies of Dipsacales sensu APG (1998; without Adoxaceae) are the presence of an amoeboid tapetum (but also present in *Adoxa*; Weberling & Hillebrand, 1986) and a nectary consisting of unicellular trichomes associated with the corolla (Wagenitz & Laing, 1984).

All analyses, separate and combined, agree with results by Downie and Palmer (1992) and Backlund and Bremer (1997), with *Abelia* being more closely related to Valerianaceae and Dipsacaceae (bootstrap support of 98%, four-gene analysis, Fig. 1C) than to Caprifoliaceae, to which it formerly belonged. The removal of *Abelia* and the tribe Linnaeeae from the remaining Caprifoliaceae was proposed by Donoghue (1983) based on a reduced number of seeds, stamens, and fertile locules in the ovary and resulted in the description of the family Linnaeaceae (Backlund & Pyck, 1998). This reduction has progressed further in Dipsacaceae and Valerianaceae. Decisive characters for uniting Linnaeaceae with these two families in the analysis by Judd et al. (1994) were the reduced nectaries and ovary shape. Achenes unite *Abelia* with Dipsacaceae and Valerianaceae as opposed to berries and drupes in Caprifoliaceae including *Symphoricarpos*. Biochemistry links *Abelia* especially with Dipsacaceae: both contain rare bis-secoiridoids, like sylvestroside and related compounds (Plouvier, 1992), found outside these taxa only in Goodeniaceae, Calyceraceae, Oleaceae, and Icacinaceae (Jensen et al., 1979; Capasso et al., 1996).

Apiales included in this analysis are *Griselinia*, *Melanophylla*, and members of Apiaceae, Araliaceae, and Pittosporaceae (Fig. 1C; 100% bootstrap support). Based on both *rbc*L and *mat*K sequence data, *Toricellia* and *Aralidium* also belong in this clade (Plunkett et al., 1997). Relationships within the Apiales are congruent with those found by Plunkett et al. (1996, 1997) based on *mat*K and *rbc*L, with broader sampling. In this circumscription, the Apiales include approximately 425 genera and 3250 species.

Apiales share several features with Asterales, such as essential oils and resins often in schizogenous oil canals, polyacetylenes, sapogenins, and the lack of true tannins (Hegnauer, 1971). Cytologically, Pittosporaceae, Araliaceae, and *Toricellia* share a base chromosome number of $x = 12$ with *Escallonia*. *Griselinia* differs in this respect with $x = 9$, a value found commonly in the euasterids II (many Asterales and Adoxaceae).

The present study provides the opportunity to evaluate ancestral character states within Apiales. Plunkett et al. (1996) suggested bicarpellate flowers and simple leaves as ancestral within Apiales. Both characters occur in Stylidiaceae and Donatiaceae, which occupy basal branches in the Asterales. Escalloniaceae and Adoxaceae have two to five carpels and mostly simple leaves, especially *Viburnum*, or a tendency to compound leaves. Our analysis is therefore consistent with earlier hypotheses (Plunkett et al., 1996).

The position of *Berzelia* is extremely uncertain, with 18S rDNA placing it even outside the euasterids II on a basal branch of euasterids I. With both *atp*B and *ndh*F, it is sister to *Escallonia*, while in the *rbc*L analysis, it is sister to *Sambucus/Viburnum*. Olmstead et al. (1993) found *Berzelia* to be sister to Dipsacales based on *rbc*L data. Improved taxon sampling within the Escalloniaceae seems especially warranted.

Conclusions

This study is another step in elucidating the phylogeny of asterids. Phylogenetic trees are largely congruent with previous results (Olmstead et al., 1992, 1993; Chase et al., 1993; Soltis et al., 1997a). The same four major clades (Asterid I–IV sensu Chase et al., 1993; Cornales, Ericales, euasterids I and II sensu APG, 1998) have been recovered; however, this analysis finds increased support for the major clades of asterids. Bootstrap values greater than 50% authenticate Cornales, Ericales, and two subclades of both euasterids I and euasterids II. In addition, the relationships of a few poorly known genera within the asterids were clarified in these analyses. Alliance of *Hydrostachys* with Hydrangeaceae (Hempel et al., 1995) was confirmed, as was the sister-group relationship of *Eremosyne* and *Escallonia* (Hibsch-Jetter et al., 1997). *Montinia* and *Hydrolea* (Hydrophyllaceae) constitute an early diverging clade of Solanales (Fig. 1B). At least parts of Icacinaceae are related to Aquifoliaceae, *Helwingia*, and *Phyllonoma* (Fig. 1C).

This study reinforces the strategy of combining multiple molecular data sets for phylogeny reconstruction. The increased number of characters leads to increased resolution and support for the revealed clades (but see Mason-Gamer & Kellogg, 1996) and faster computer run times, allowing more thorough searches. The continued combination of multiple genes in phylogenetic analyses will help us learn more about both organismal and molecular evolution.

Literature Cited

Albach, D. C., D. E. Soltis, M. W. Chase & P. S. Soltis. In press a. Phylogenetic placement of the enigmatic angiosperm *Hydrostachys*. Taxon.

———, P. S. Soltis, & D. E. Soltis. In press b. Patterns of embryological evolution in the asterids. Syst. Bot.

Albert, V. A., S. E. Williams & M. W. Chase. 1992. Carnivorous plants: Phylogeny and structural evolution. Science 257: 1491–1494.

Al-Shammary, K. I. 1991. Systematic Studies of the Saxifragaceae, Chiefly from the Southern Hemisphere. Ph.D. Dissertation, University of Leicester.

——— & R. J. Gornall. 1994. Trichome anatomy of the Saxifragaceeae s.l. from the southern hemispheres. Bot. J. Linn. Soc. 114: 99–131.

Anderberg, A. A. 1993. Cladistic interrelationships and major clades of the Ericales. Pl. Syst. Evol. 184: 207–231.

——— & B. Stahl. 1995. Phylogenetic interrelationships in the order Primulales, with special emphasis on the family circumscription. Canad. J. Bot. 73: 1699–1730.

———, ——— & M. Källersjö. 1998. Phylogenetic relationships in the Primulales inferred from *rbc*L sequence data. Pl. Syst. Evol. 211: 93–102.

APG (Angiosperm Phylogeny Group). 1998. An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85: 531–553.

Backlund, A. & B. Bremer. 1997. Phylogeny of the Asteridae s. str. based on *rbc*L sequences, with particular reference to the Dipsacales. Pl. Syst. Evol. 207: 225–254.

——— & N. Pyck. 1998. Diervillaceae and Linnaeaceae, two new families of caprifolioids. Taxon 47: 657–661.

Baillon, H. 1867–1895. Histoire des Plantes. Paris.

Bate-Smith, E. C., I. K. Ferguson, K. Hutson, S. R. Jensen, B. J. Nielsen & T. Swain. 1975. Phytochemical interrelationships in the Cornaceae. Biochem. Syst. Ecol. 3: 79–89.

Boros, C. A. & F. R. Stermitz. 1991. Iridoids. An updated review, Part II. J. Nat. Prod. 54: 1173–1246.

Bremer, B., R. G. Olmstead, L. Struwe & J. A. Sweere. 1994. *rbc*L sequences support exclusion of *Retzia*, *Desfontainia*, and *Nicodemia* from the Gentianales. Pl. Syst. Evol. 190: 213–230.

Breuer, B., T. Stuhlfauth, H. Fock & H. Huber. 1987. Fatty acids of some Cornaceae, Hydrangeaceae, Aquifoliaceae, Hamamelidaceae and Styracaceae. Phytochemistry 26: 1441–1445.

Bult, C., M. Källersjö & Y. Suh. 1992. Amplification and sequencing of 16/18S rDNA from gel-purified total plant DNA. Pl. Molec. Biol. Reporter 10: 273–284.

Cantino, P. D. 1992. Evidence for a polyphyletic origin of the Labiatae. Ann. Missouri Bot. Gard. 79: 361–379.

Capasso, A., R. Urrunaga, L. Garofala, L. Sorrentino & R. Aquino. 1996. Phytochemical and pharmacological studies on medicinal herb *Acicarpha tribuloides*. Int. J. Pharmacognosy 34: 255–261.

Carlquist, S. 1992. Wood anatomy of sympetalous dicotyledon families: A summary, with comments on systematic relationships and evolution of the woody habit. Ann. Missouri Bot. Gard. 79: 303–332.

Changzeng, W. & Y. Dequan. 1997. Diterpenoid, sesquiterpenoid and secoiridoid glucosides from *Aster auriculatus*. Phytochemistry 45: 1483–1487.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y.-L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedrén, B. S. Gaut, R. K. Jansen, K.-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q.-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan & V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.

Cosner, M. E., R. K. Jansen & T. G. Lammers. 1994. Phylogenetic relationships in the Campanulales based on *rbc*L sequences. Pl. Syst. Evol. 190: 79–95.

Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia Univ. Press, New York.

Cullings, K. W. 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. Molec. Ecol. 1: 233–240.

Dahlgren, R. 1975. The distribution of characters within an angiosperm system I. Some embryological characters. 1975. Bot. Not. 128: 181–197.

———, S. Rosendal-Jensen & B. J. Nielsen. 1981. A revised classification of the angiosperms with comments on correlation between chemical and other characters. Pp. 149–204 *in* D. A. Young & D. S. Seigler (editors), Phytochemistry and Angiosperm Phylogeny. Praeger Publishers, New York.

Dickinson, T. A. & R. Sattler. 1974. Development of the epiphyllous inflorescence of *Phyllonoma integerrima* (Turcz.) Loes.: Implications for comparative morphology. Bot. J. Linn. Soc. 69: 1–13.

Dickison, W. C. 1993. Floral anatomy of the Styracaceae, including observations on intra-ovarian trichomes. Bot. J. Linn. Soc. 112: 223–255.

Donoghue, M. J. 1983. The phylogenetic relationships of *Viburnum*. Pp. 143–166 *in* N. Platnick & V. A. Funk (editors), Advances in Cladistics, Vol. 2. Columbia Univ. Press, New York.

Downie, S. R. & J. D. Palmer. 1992. Restriction site mapping of the chloroplast DNA inverted repeat: A molecular phylogeny of the Asteridae. Ann. Missouri Bot. Gard. 79: 266–283.

Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

El-Naggar, L. J. & J. L. Beal. 1980. Iridoids. A review. J. Nat. Prod. 43: 649–707.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy. Almqvist & Wiksell, Stockholm.

Eyde, R. H. 1964. Inferior ovary and generic affinities of *Garrya*. Amer. J. Bot. 51: 1083–1092.

———. 1988. Comprehending *Cornus*: Puzzles and progress in the systematics of dogwood. Bot. Rev. 54: 233–351.

Farris, J. S., V. A. Albert, M. Källersjö, D. Lipscomb & A. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12: 99–124.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Ferguson, I. K. 1977. Cornaceae. World Pollen and Spore Flora 6: 1–34.

Goldblatt, P. (editor). 1985. Index to Plant Chromosome Numbers 1982–1983. Monogr. Syst. Bot. Missouri Bot. Gard. Vol. 13.

Gray, A. 1875. A conspectus of the North American Hydrophyllaceae. Proc. Amer. Acad. 10: 312–332.

Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47: 9–17.

Gustafsson, M. H. G. 1995. Petal venation in the Asterales and related orders. Bot. J. Linn. Soc. 118: 1–18.

——— & K. Bremer. 1997. The circumscription and systematic position of Carpodetaceae. Austral. Syst. Bot. 10: 855–862.

———, A. Backlund & B. Bremer. 1996. Phylogeny of the Asterales sensu lato based on *rbc*L sequences with particular reference to the Goodeniaceae. Pl. Syst. Evol. 199: 217–242.

Hamby, K. R. & E. A. Zimmer. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. Pp. 50–91 *in* P. S. Soltis, D. E. Soltis & J. J. Doyle, Molecular Systematics of Plants. Chapman and Hall, New York.

Hansen, H. V. 1992. Studies in the Calyceraceae with a discussion of its relationships to Compositae. Nordic J. Bot. 12: 63–75.

Hegnauer, R. 1971. Chemical patterns and relationships of Umbelliferae. *In:* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae, Supplement 1. Bot. J. Linn. Soc. 64: 267–277.

———. 1990. Chemotaxonomie der Pflanzen. Band 9. Birkhäuser Verlag, Basel, Boston, Berlin.

——— & M. Hegnauer. 1992. Chemotaxonomie der Pflanzen. Band 10. Birkhäuser Verlag, Basel, Boston, Berlin.

Hempel, A. L., P. A. Reeves, R. G. Olmstead & R. K. Jansen. 1995. Implications of *rbc*L sequence data for higher order relationships of the Loasaceae and the anomalous aquatic plant *Hydrostachys* (Hydrostachyaceae). Pl. Syst. Evol. 194: 25–37.

Henrickson, J. 1967. Pollen morphology of the Fouquieriaceae. Aliso 6: 137–160.

Hibsch-Jetter, C., D. E. Soltis & T. D. MacFarlane. 1997. Phylogenetic analysis of *Eremosyne pectinata* (Saxifragaceae s.l.) based on *rbc*L sequence data. Pl. Syst. Evol. 204: 225–232.

Hillis, D. M. 1996. Inferring complex phylogenies. Nature 383: 130–131.

———. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. Syst. Biol. 47: 3–8.

Hoot, S. B., A. Culham & P. R. Crane. 1995. The utility of *atp*B gene sequences in resolving phylogenetic relationships: Comparison with *rbc*L and 18S Ribosomal DNA sequences in the Lardizabalaceae. Ann. Missouri Bot. Gard. 82: 194–207.

Hufford, L. 1992. Rosidae and their relationships to other nonmagnoliid dicotyledons: A phylogenetic analysis us-

ing morphological and chemical data. Ann. Missouri Bot. Gard. 79: 218–248.

Jensen, S. R. 1991. Plant iridoids, their biosynthesis and distribution in angiosperms. Pp. 133–158 *in* J. B. Harborne & F. A. Tomas-Barberan (editors), Ecological Chemistry and Biochemistry of Plant Terpenoids. Clarendon Press, Oxford.

———. 1992. Systematic implications of the distribution of iridoids and other chemical compounds in the Loganiaceae and other families of the Asteridae. Ann. Missouri Bot. Gard. 79: 284–302.

———, B. J. Nielsen & R. Dahlgren. 1975. Iridoid compounds, their occurrence and systematic importance in the angiosperms. Bot. Not. 128: 148–180.

———, S. E. Lyse-Petersen & B. J. Nielsen. 1979. Novel bis-iridoid glucosides from *Dipsacus sylvestris*. Phytochemistry 18: 273–277.

———, L. Ravnkilde & J. Schripsema. 1998. Undedoside derivatives in *Nuxia* and their biosynthesis. Phytochemistry 47: 1007–1011.

Johnson, L. A., D. E. Soltis & P. S. Soltis. 1999. Phylogenetic relationships of Polemoniaceae inferred from 18S ribosomal DNA sequences. Pl. Syst. Evol. 214: 65–89.

———, J. L. Schultz, D. E. Soltis & P. S. Soltis. 1996. Monophyly and generic relationships of Polemoniaceae based on *mat*K sequences. Amer. J. Bot. 83: 1207–1224.

Judd, W. S., R. W. Sanders & M. J. Donoghue. 1994. Angiosperm family pairs: Preliminary phylogenetic analyses. Harvard Pap. Bot. 5: 1–51.

Jussieu, A. L. de. 1789. Genera plantarum secundum ordines naturales disposita, . . . Herissant & Barrois, Paris.

Källersjö, M., J. S. Farris, M. W. Chase, B. Bremer, M. F. Fay, C. J. Humphries, G. Petersen, O. Seberg & K. Bremer. 1998. Simultaneous parsimony jackknife analysis of 2538 *rbc*L DNA sequences reveals support for major clades of green plants, land plants, seed plants and flowering plants. Pl. Syst. Evol. 213: 259–287.

Kaplan, M. A. C., J. Ribeiro & O. R. Gottlieb. 1991. Chemogeographical evolution of terpenoids in Icacinaceae. Phytochemistry 30: 2671–2676.

Karehed, J., J. Lundberg, B. Bremer & K. Bremer. 1999. Evolution of the Australasian families Alseuosmiaceae, Argophyllaceae, and Phellinaceae. Syst. Bot. 24: 660–682.

Keller, J. A., P. S. Herendeen & P. R. Crane. 1996. Fossil flowers and fruits of the Actinidiaceae from the Campanian (Late Cretaceous) of Georgia. Amer. J. Bot. 83: 528–541.

Kim, K. J. & R. K. Jansen. 1995. *ndh*F sequence evolution and the major clades in the sunflower family. Proc. Natl. Acad. Sci. U.S.A. 92: 10379–10383.

Koontz, J. A. & D. E. Soltis. 1999. DNA sequence data reveal polyphyly of Brexioideae (Brexiaceae; Saxifragaceae sensu lato). Pl. Syst. Evol. 219: 199–208.

Kron, K. A. 1996. Phylogenetic relationships of Empetraceae, Epacridaceae, Ericaceae, Monotropaceae, and Pyrolaceae: Evidence from nuclear ribosomal 18S sequence data. Ann. Bot. 77: 293–303.

——— & M. W. Chase. 1993. Systematics of the Ericaceae, Empetraceae, Epacridaceae and related taxa based upon *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 735–741.

Lammers, T. G. 1992. Circumscription and phylogeny of the Campanulales. Ann. Missouri Bot. Gard. 81: 388–413.

Maddison, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst. Zool. 40: 315–328.

Maddison, W. & D. R. Maddison. 1992. MacClade: Analysis of Phylogeny and Character Evolution. Version 3.04. Sinauer, Sunderland, Massachusetts.

Mason-Gamer, R. J. & E. A. Kellogg. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. 45: 524–545.

Mishler, B. D., L. A. Lewis, M. A. Buchheim, K. S. Renzaglia, D. G. Garbary, C. F. Delwiche, F. W. Zechman, T. S. Kantz & R. L. Chapman. 1994. Phylogenetic relationships of the "Green Algae" and "Bryophytes." Ann. Missouri Bot. Gard. 81: 451–483.

Mølgaard, P. 1985. Caffeic acid as a taxonomic marker in dicotyledons. Nordic J. Bot. 5: 203–213.

Morgan, D. R. & D. E. Soltis. 1993. Phylogenetic relationships among members of Saxifragaceae sensu lato based on *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 631–660.

Mort, M. E., P. S. Soltis, D. E. Soltis & M. L. Mabry. 2000. Comparison of three methods for estimating internal support on phylogenetic trees. Syst. Biol. 49: 160–171.

Morton, C. M., M. W. Chase, K. A. Kron & S. M. Swensen. 1996. A molecular evaluation of the monophyly of the order Ebenales based upon *rbc*L sequence data. Syst. Bot. 21: 567–586.

———, S. A. Mori, G. T. Prance, K. G. Karol & M. W. Chase. 1997. Phylogenetic relationships of Lecythidaceae: A cladistic analysis using *rbc*L sequence and morphological data. Amer. J. Bot. 84: 530–540.

Nash, G. V. 1903. A revision of the family Fouquieriaceae. Bull. Torrey Bot. Club 30: 449–459.

Naylor, G. J. P. & W. M. Brown. 1998. Amphioxus mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparisons of sequences. Syst. Biol. 47: 61–76.

Nickrent, D. L. & C. R. Franchina. 1990. Phylogenetic relationships of the Santalales and relatives. J. Molec. Evol. 31: 294–301.

——— & D. E. Soltis. 1995. A comparison of angiosperm phylogenies from nuclear 18S rDNA and *rbc*L sequences. Ann. Missouri Bot. Gard. 82: 208–234.

Nishino, E. 1978. Corolla tube formation in four species of Solanaceae. Bot. Mag. Tokyo 91: 263–277.

———. 1983. Corolla tube formation in the Primulaceae and Ericales. Bot. Mag. Tokyo 96: 319–342.

Nixon, K. 1999. The parsimony ratchet: A rapid means for analyzing large data sets. Cladistics 15: 407–414.

Ohtani, T., H. Uchimiya, A. Kato, H. Harada, M. Sugita & M. Sugiura. 1984. Location and nucleotide sequence of a tobacco chloroplast DNA segment capable of replication in yeast. Molec. Gen. Genet. 195: 1–4.

Olmstead, R. G. & P. A. Reeves. 1995. Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbc*L and *ndh*F sequences. Ann. Missouri Bot. Gard. 82: 176–193.

——— & J. A. Sweere. 1994. Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. Syst. Biol. 43: 467–481.

———, H. J. Michaels, K. M. Scott & J. D. Palmer. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbc*L. Ann. Missouri Bot. Gard. 79: 249–265.

———, B. Bremer, K. M. Scott & J. D. Palmer. 1993. A parsimony analysis of the Asteridae s.l. based on *rbc*L sequences. Ann. Missouri Bot. Gard. 80: 700–722.

———, R. K. Jansen, K.-J. Kim & S. J. Wagstaff. 2000. The phylogeny of the Asteridae s.l. based on chloroplast *ndh*F sequences. Molec. Phylogenet. Evol. 16: 96–112.

Oxelman, B., M. Backlund & B. Bremer. 1999. Relationships of the Buddlejaceae s.l. investigated using parsimony jackknife and branch support analysis of chloroplast *ndh*F and *rbc*L sequence data. Syst. Bot. 24: 164–182.

Plouvier, V. 1992. Chimiotaxinomie des Caprifoliaceae et relations avec quelques familles voisines. Bull. Mus. natl. Hist. nat., sect. B. Adansonia 14: 461–472.

——— & J. Favre-Bonvin. 1971. Les iridoides et secoiridoides: Repartition, structure, propriétés, biosynthèse. Phytochemistry 10: 1697–1722.

Plunkett, G. 1994. A Molecular-Phylogenetic Approach to the "Family-Pair Dilemma" in Apiales and Cyperales. Ph.D. Thesis, Washington State University.

———, D. E. Soltis & P. S. Soltis. 1996. Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbc*L. Amer. J. Bot. 83: 499–515.

———, ——— & P. S. Soltis. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbc*L sequence data. Amer. J. Bot. 84: 565–580.

Poe, S. & D. L. Swofford. 1999. Taxon sampling revisited. Nature 398: 299–300.

Poser, G. L. von, M. E. Toffoli, M. Sobral & A. T. Heniques. 1997. Iridoid glucosides substitution patterns in Verbenaceae and their taxonomic implication. Pl. Syst. Evol. 205: 265–287.

Quibell, C. F. 1972. Comparative and Systematic Anatomy of Carpenterieae (Philadelphiaceae). Ph.D. Dissertation, University of California, Berkeley.

Rahn, K. 1996. A phylogenetic study of the Plantaginaceae. Bot. J. Linn. Soc. 120: 145–198.

Raven, P. H. 1975. The bases of angiosperm phylogeny: Cytology. Ann. Missouri Bot. Gard. 62: 724–764.

Reichenbach, H. G. L. 1827–1829. Dr. Joh. Christ. Moessler's Handbuch der Gewaechskunde, Ed. 2, 3 Vols. Hammerich, Altona.

Rice, K. A., M. J. Donoghue & R. G. Olmstead. 1997. Analyzing large data sets. Syst. Biol. 46: 554–563.

Roels, P. & E. Smets. 1996. A floral ontogenetic study in the Dipsacales. Int. J. Pl. Sci. 157: 203–218.

———, L. P. Ronse Decraene & E. F. Smets. 1997. A floral ontogenetic investigation of the Hydrangeaceae. Nordic J. Bot. 17: 235–254.

Romeike, A. 1978. Tropane alkaloids—Occurrence and systematic importance in angiosperms. Bot. Not. 131: 85–96.

Savolainen, V., M. W. Chase, S. B. Hoot, C. M. Morton, D. E. Soltis, C. Bayer, M. F. Fay, A. Y. de Bruijn, S. Sullivan & Y.-L. Qiu. 2000. Phylogenetics of flowering plants based upon a combined analysis of plastid *atp*B and *rbc*L gene sequences. Syst. Biol. 49: 306–362.

Scogin, R. 1977. Anthocyanins of the Fouquieriaceae. Biochem. Syst. & Ecol. 5: 265–267.

———. 1978. Leaf phenolics of the Fouquieriaceae. Biochem. Syst. & Ecol. 6: 297–298.

Scotland, R. W., J. A. Sweere, P. A. Reeves & R. G. Olmstead. 1995. Higher level systematics of Acanthaceae determined by chloroplast DNA sequences. Amer. J. Bot. 82: 266–275.

Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.

Skvarla, J. J., B. L. Turner, V. C. Patel & A. S. Tomb. 1977. Pollen morphology in the Compositae and in morphologically related families. Pp. 141–201 *in* V. H. Heywood, J. B. Harborne & B. L. Turner, The Biology and Chemistry of the Compositae, Vol.1. Academic Press, London, New York, San Francisco.

Soltis, D. E. & P. S. Soltis. 1997. Phylogenetic relationships in Saxifragaceae sensu lato: A comparison of topologies based on 18S rDNA and *rbc*L sequences. Amer. J. Bot. 84: 504–522.

———, Q.-Y. Xiang & L. Hufford. 1995. Relationships and evolution of Hydrangeaceae based on *rbc*L sequence data. Amer. J. Bot. 82: 504–514.

———, ———, D. L. Nickrent, L. A. Johnson, W. J. Hahn, S. B. Hoot, J. A. Sweere, R. K. Kuzoff, K. A. Kron, M. W. Chase, S. M. Swensen, E. A. Zimmer, S.-M. Chaw, L. J. Gillespie, W. J. Kress & K. J. Sytsma. 1997a. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. Ann. Missouri Bot. Gard. 84: 1–49.

———, C. Hibsch-Jetter, P. S. Soltis, M. W. Chase & J. Farris. 1997b. Molecular phylogenetic relationships among angiosperms: An overview based on *rbc*L and 18S rDNA sequences. Pp. 157–178 *in* K. Iwatsuki & P. H. Raven (editors), Evolution and Diversification of Land Plants. Springer-Verlag, Tokyo.

———, P. S. Soltis, M. Mort., M. W. Chase, V. Savolainen, S. B. Hoot & C. M. Morton. 1998. Inferring complex phylogenies: An empirical approach using three large DNA data sets for angiosperms. Syst. Biol. 47: 32–42.

———, ———, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanis, V. Savolainen, W. H. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, K. C. Nixon & J. S. Farris. 2000. Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbc*L, and *atp*B sequences. Bot. J. Linn. Soc. 133: 381–461.

Soltis, P. S. & D. E. Soltis. 1996. Phylogenetic analysis of large molecular data sets. Bol. Soc. Bot. Mexicana 59: 99–113.

——— & ———. 1998. Molecular evolution of 18S rDNA in angiosperms: Implications for character weighting in phylogenetic analysis. Pp. 188–210 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II. Kluwer, Norwell, Massachusetts.

Stebbins, G. L. 1971. Flowering Plants. Harvard Univ. Press, Cambridge, Massachusetts.

Struwe, L., V. A. Albert & B. Bremer. 1994. Cladistics and family level classification of the Gentianales. Cladistics 10: 175–206.

Stuhlfauth, T., H. Fock, H. Huber & K. Klug. 1985. The distribution of fatty acids including petroselinic and tariric acids in the fruit and seed oils of the Pittosporaceae, Araliaceae, Umbelliferae, Simarubaceae and Rutaceae. Phytochemistry 13: 147–153.

Swofford, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (and other methods) beta vers. 4.0. Sinauer, Sunderland, Massachusetts.

——— & G. J. Olsen. 1990. Phylogeny reconstruction. Pp. 411–501 *in* D. M. Hillis & C. Moritz. Molecular Systematics. Sinauer, Sunderland, Massachusetts.

Takhtajan, A. 1964. The taxa of the higher plants above the rank of order. Taxon 13: 160–164.

———. 1980. Outline of the classification of flowering plants (Magnoliophyta). Bot. Rev. 46: 225–359.
———. 1997. Diversity and Classification of Flowering Plants. Columbia Univ. Press, New York.
Thorne, R. F. 1992. Classification and geography of the flowering plants. Bot. Rev. 58: 225–348.
Tobe, H. & N. R. Morin. 1996. Embryology and circumscription of Campanulaceae and Campanulales: A review of literature. J. Pl. Res. 109: 425–435.
Tsou, C.-H. 1995. Embryology of Theaceae—Anther and ovule development of *Adinandra*, *Cleyera* and *Eurya*. J. Pl. Res. 108: 77–86.
Varadaraj, K. & D. M. Skinner. 1994. Denaturants or cosolvents improve the specificity of PCR amplification of a G+C-rich DNA using genetically-engineered DNA polymerases. Gene 140: 1–5.
Vawter, L. & W. M. Brown. 1993. Rates and patterns of base change in the small subunit ribosomal RNA gene. Genetics 134: 597–608.
Wagenitz, G. 1992. The Asteridae: Evolution of a concept and its present status. Ann. Missouri Bot. Gard. 79: 209–217.
——— & B. Laing. 1984. Die Nektarien der Dipsacales und ihre systematische Bedeutung. Bot. Jahrb. Syst. 104: 483–507.
Wagstaff, S. J. & R. G. Olmstead. 1997. Phylogeny of Labiatae and Verbenaceae inferred from *rbc*L sequences. Syst. Bot. 22: 165–177.
———, L. Hickerson, R. Spangler, P. A. Reeves & R. G. Olmstead. 1998. Phylogeny in Labiatae s.l., inferred from cpDNA sequences. Pl. Syst. Evol. 209: 265–274.
Warming, E. 1879. Haandbog I den systematiske botanik. P. G. Philipsens Forlag, Kjobenhaven.
Weberling, F. & M. Hillebrand. 1986. Further studies on tapetum development in Caprifoliaceae. Beitr. Biol. Pflanzen. 61: 3–20.
Wolf, P. G., K. M. Pryer, A. R. Smith & M. Hasebe. 1998. Phylogenetic studies of extant pteridophytes. Pp. 541–556 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II. Kluwer, Norwell, Massachusetts.
Xiang, Q.-Y., D. E. Soltis, D. R. Morgan & P. S. Soltis. 1993. Phylogenetic relationships of *Cornus* L. sensu lato and putative relatives infrerred from *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 723–734.
———, D. E. Soltis & P. S. Soltis. 1998. Phylogenetic relationships of Cornaceae and close relatives inferred from *mat*K and *rbc*L sequences. Amer. J. Bot. 85: 285–297.
Yamazaki, T. 1974. A system of gamopetalae based on the embryology. J. Fac. Sci. Univ. Tokyo, III 11: 263–281.
Yang, Z. 1998. On the best evolutionary rate for phylogenetic analysis. Syst. Biol. 47: 125–133.
Zurawski, G. & M. T. Clegg. 1993. *rbc*L sequence data and phylogenetic reconstruction in seed plants: Foreword. Ann. Missouri Bot. Gard. 80: 523–525.

Appendix 1. Sequences of 18S rDNA, *rbc*L, *atp*B, and *ndh*F used in the analyses together with their GenBank number. GenBank numbers with an asterisk mark previously unpublished sequences (for vouchers see Appendix 2). Family names follow the Angiosperm Phylogeny Group, 1998. Taxa together in one row are those grouped for the combined analysis.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
ACANTHACEAE							
Barleria prionitis L.	AF107567*	*B. prionitis*	L01886	*B. prionitis*	AJ236179*	*B. prionitis*	U12653
Justicia americana (L.) Vahl	AF107568*	*J. americana*	L14401	*J. americana*	AJ236178*	*J. americana*	U12663
Thunbergia alata Sims	AF107569*	*T. usambarica* Lindau	L12596	*T. coccinea* Wall.	AF235625	*T. alata*	U12667
ACTINIDIACEAE							
Actinidia arguta (Siebold & Zucc.) Miq.	U42495	*A. chinensis* Planch.	L01882	*A. chinensis*	AJ235382	*A. arguta*	AJ236238*
ADOXACEAE							
Sambucus ebulus L.	AJ236005*	*S. racemosa* L.	L14066	*S. nigra* L.	AJ235591	*S. canadensis*	AF130196
Viburnum acerifolia L.	AJ236007*	*V. acerifolia*	L01959	*V. opulus* L.	AJ235640	*V. sargentii* Koehne	AF130197
ALSEUOSMIACEAE							
Alseuosmia macrophylla Cunn.	AF206844	*A. macrophylla*	X87377	*A. macrophylla*	AJ236198*	*A. macrophylla*	AF236239*
APIACEAE							
Lomatium triternatum (Pursh) Coult. & Rose	U42498	*Coriandrum sativum* L.	L11676	*Apium graveolens* L.	AJ235396	*C. sativum*	AF130199
APOCYNACEAE							
Alstonia scholaris (L.) R. Br.	AF107570*	*A. scholaris*	X91760				
Apocynum cannabinum L.	AF107571*	*A. cannabinum*	L11678				
Nerium oleander L.	AF107572*	*N. oleander*	AF156735*	*N. oleander*	AJ236189*	*N. oleander*	AF130168
Tabernaemontana sp.	U42499	*Thevetia peruviana* (Pers.) Schumann	X91773	*Plumeria obtusa* L.	AF235566	*T. peruviana*	AF130196
AQUIFOLIACEAE							
Ilex opaca Soland.	AF161008*	*I. crenata* Thunb.	L01928	*I. crenata*	AJ235502	*I. crenata*	AF130206
ARALIACEAE							
Delarbrea micheana (F. v. Muell.) F. v. Muell.	AF107573*	*D. micheana*	U50243	*D. micheana*	AJ236211*	*D. micheana*	AJ236240*
Hedera helix L.	U42500	*H. helix*	L01924	*H. helix*	AJ235488	*H. helix*	AF130203
Panax quinquefolius L.	AF107574*	*P. quinquefolius*	U50250	*P. quinquefolius*	AJ236210*	*P. quinquefolius*	AF130204
ARGOPHYLLACEAE							
Corokia cotoneaster Raoul	U42523	*C. cotoneaster*	L11221	*C. cotoneaster*	AJ235445	*C. cotoneaster*	AF131082
ASTERACEAE							
Barnadesia caryophyllea (Vell.) S. F. Blake	AF107575*	*B. caryophyllea*	L01887			*B. caryophyllea*	L39394

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
Gerbera jamesonii Bolus	AF107576*	*G. jamesonii*	L13643	*G. jamesonii*	AJ236200*	*G. jamesonii*	L39403
Helianthus annuus L.	AF107577*	*H. annuus*	L13929	*H. annuus*	AJ236205*	*H. annuus*	L39383
Tagetes sp.	U42501	*T. erecta* L.	L13637	*Tagetus* sp.	AJ236206*	*T. erecta*	L39466
Tragopogon dubius Scop.	U42502	*T. porrifolius* L.	L13647	*T. dubius*	AJ236197*	*T. porrifolius*	L19391
AUCUBACEAE							
Aucuba japonica Thunb.	U42522	*A. japonica*	L11210	*A. japonica*	AJ235402		
BALSAMINACEAE							
Impatiens wallerana J. D. Hook	L49285	*I. capensis* Meerb.	Z83142	*I. repens* Moon	AJ235503	*I. biflora* Willd.	AF130210
BIGNONIACEAE							
Campsis radicans (L.) Bureau	AF107578*	*C. radicans*	AF156737*	*C. radicans*	AJ236168*	*C. radicans*	AF130144
Catalpa bignonioides Walter	AF107579*	*C. bignonioides*	L11679	*C. bignonioides*	AJ235428	*Catalpa* sp.	L36397
BORAGINACEAE							
Borago officinalis L.	AF107580*	*B. officinalis*	L11680	*B. officinalis*	AJ235414	*B. officinalis*	L36393
Bourreria succulenta Jacq.	U38319	*B. virgata* (Swartz.) G. Don	AF258345				
Hydrophyllum fendleri (A. Gray) Heller	AJ236019*	*H. virginianum* L.	L01927	*H. canadense* L.	AJ235498	*H. virginianum*	AF019646*
Phacelia bicolor Pursh	L49292	*P. heterophylla* Torr.	AF258344			*P. patuliflora* (Engelm. & A. Gray) A. Gray	AF10179
BRUNIACEAE							
Berzelia lanuginosa (L.) Brongn.	U42508	*B. lanuginosa*	L14391	*B. lanuginosa*	AJ236218*	*B. lanuginosa*	AJ236241*
BUDDLEJACEAE							
Buddleja davidii Franch.	AF107581*	*B. davidii*	L14392	*B. auriculata* Benth.	AJ235420	*B. davidii*	L39394
BYBLIDACEAE							
Byblis gigantea Lindl.	U42590	*B. liniflora* Salisb.	L01891	*B. gigantea*	AJ236181*		
CALYCERACEAE							
Boopis graminea Phil.	AF107583*	*B. anthemoides* Juss.	L13860	*B. graminea*	AJ236199*	*B. anthemoides*	L39384
CAMPANULACEAE							
Campanula ramosa Wall.	U42510	*C. ramosa*	L13861	*C. trachelium* L.	AJ235423	*C. ramosa*	L39387
Codonopsis pilosula (Franch.) Nannf.	AF107584*	*C. ovata* Benth.	L18797	*C. pilosula*	AJ236203*	*C. lanceolata* (Siebold & Zucc.) Trautv.	AF130185
Lobelia erinus L.	U42785	*L. erinus*	L01931	*L. angulata* Forst f.	AJ235524	*L. cardinalis* L.	AF130187
CAPRIFOLIACEAE							
Lonicera maackii (Rupr.) Maxim.	U66701	*L. orientalis* Lam	X87389			*L. japonica* Thunb.	AF13094

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
Symphoricarpus albus (L.) Blake	U42513	*S. albus*	L11682	*S. albus*	AJ236208*	*S. orbiculatus* Moench.	AF130195
CLETHRACEAE							
Clethra alnifolia L.	U42521	*C. alnifolia*	L12609	*C. arborea* Soland.	AJ235438	*C. alnifolia*	AJ236242*
CONVOLVULACEAE							
Convolvulus arvensis L.	AJ236013*	*C. tricolor* L.	L11683	*C. arvensis*	AJ236186*	*C. arvensis*	AJ236243*
Ipomoea hederacea Jacq.	U38310	*I. coccinea* L.	L14400	*I. mauritiana* Jacq.	AJ235505	*I. hederacea*	U08918
CORNACEAE							
Alangium chinense (Lour.) Harms	AF206843	*A. chinense*	L11209	*A. platanifolium* (Siebold & Zucc.) Harms	AJ235386	*A. platanifolium*	AF130221
Cornus officinalis Siebold & Zucc.	U52033	*C. officinalis*	L11219	*C. officinalis*	AJ236229*	*C. florida* L.	AF130220
Camptotheca acuminata Decne.	U42789	*C. acuminata*	L11211				
Davidia involucrata Baill.	AJ235991*	*D. involucrata*	L11223			*D. involucrata*	AJ236265*
Nyssa ogeche Marshall	U52032	*N. ogeche*	L11228	*N. sylvatica* Marshall	AJ235545	*N. ogeche*	AJ236266*
CYRILLACEAE							
Cyrilla racemiflora L.	U43494	*C. racemiflora*	L01900	*C. racemiflora*	AJ235449		
DIAPENSIACEAE							
Diapensia lapponica L.	L49278	*D. lapponica*	L12612				
Galax urceolata (Poir.) Brummitt	L49281	*G. aphylla* L.	Z80184	*G. urceolata*	AJ236223*	*G. urceolata*	AJ236245*
DIPSACACEAE							
Dipsacus sp.	U43150	*D. sativus* (L.) Honck.	L13824				
Scabiosa sp.	AJ236006*	*Scabiosa* sp.	AF156734*	*Scabiosa* sp.	AJ236207*	*S. mansenensis* Nakai	AF130184
DONATIACEAE							
Donatia sp.	AJ236012*	*D. fascicularis* J. R. Forst. & G. Forst.	X87385	*Donatia* sp.	AJ236203*	*Donatia* sp.	AJ236246*
EBENACEAE							
Diospyros virginiana L.	U43295	*D. virginiana*	L12613	*D. kaki* L.	AJ235457	*D. texana* Scheele	AF130213
EREMOSYNACEAE							
Eremosyne pectinata Endl.	U42807	*E. pectinata*	L47969	*E. pectinata*	AJ236215*	*E. pectinata*	AJ236272*
ERICACEAE							
Arbutus unedo L.	AF161008*	*A. canariensis* Duhamel	L12597	*A. unedo*	AF266738	*A. unedo*	AJ236247*
Arctostaphylos uva-ursi (L.) Spreng.	L49272	*A. uva-ursi*	L12598	*A. uva-ursi*	AJ236225*	*A. uva-ursi*	AJ236248*

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
Enkianthus campanulatus Nicholson	Kron 1996	*E. campanulatus*	L12616				
Vaccinium macrocarpon Aiton	L49297	*V. macrocarpon*	L12625				
Pyrola rotundifolia L.	Kron 1996	*P. rotundifolia*	L12622				
ESCALLONIACEAE							
Escallonia coquimbensis Remy	U42544	*E. coquimbensis*	L11183	*Escallonia* sp.	AJ235467	*E. rubra* Ruiz. & Pav.	AJ236251*
EUCOMMIACEAE							
Eucommia ulmoides Oliv.	L54066	*E. ulmoides*	L01917	*E. ulmoides*	AJ235469		
FOUQUIERIACEAE							
Fouquieria splendens Engelm.	L49280	*F. splendens*	L11675	*F. columnaris* (Kellogg) Curran	AJ235501	*F. splendens*	AJ236249*
GARRYACEAE							
Garrya elliptica Lindl.	U42540	*G. elliptica*	L01919	*G. elliptica*	AJ235478	*G. elliptica*	AF147714
GELSEMIACEAE							
Gelsemium sempervirens (L.) J. St.-Hil.	AJ236025*	*G. sempervirens*	L14397	*G. sempervirens*	AJ236193*	*G. sempervirens*	AF130170
GENTIANACEAE							
Anthocleista grandiflora Gilg	AJ236026*	*A. grandiflora*	L14389			*A. grandiflora*	AJ236250*
Exacum affine Regel	AJ236023*	*E. affine*	L11684	*E. affine*	AJ236195*	*E. affine*	AH008318
GESNERIACEAE							
Aeschynanthus radicans Jack.	U42542	*A. micranthus Clarke*	AF170222			*A. micranthus*	U62169
Cyrtandra hawaiensis Clarke	AJ236053*	*C. hawaiensis*	AF170229	*C. hawaiensis*	AJ236172*	*C. hawaiensis*	AF130159
Drymonia urceolata Wiehler	AJ236050*	*D. urceolata*	AF170232				
Nematanthus hirsutus (Mart.) Wiehler	AF161012*	*N. hirsutus*	L36446				
Rhynchoglossum notonianum (Wall.) Burtt	AJ236052*	*R. notonianum*	AF170244	*R. notonianum*	AJ236170*	*R. notonianum*	U62179
Streptocarpus holstii Engl.	AJ236038*	*S. holstii*	L14409	*Saintpaulia ionantha* Wendl.	AJ235588	*S. holstii*	L36415
Titanotrichum oldhamii (Hemsl.) Soler.	AJ236054*	*T. oldhamii*	AF206829	*T. oldhamii*	AJ236171*	*T. oldhamii*	U62187
GOODENIACEAE							
Scaevola aemula R. Br.	AJ236008*	*S. frutescens* Krause	L13932			*S. frutescens*	L39385
GRISELINIACEAE							
Griselinia littoralis (Raoul) Raoul	AJ236000*	*G. lucida* G. Forst	L11225	*G. littoralis*	AJ236213*	*G. lucida*	AF130205

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
HALESIACEAE							
Halesia carolina L.	L49284	*H. carolina*	Z80190	*H. diptera* Ellis	AJ236226*	*H. tetraptera* Ellis	AF130214
HELWINGIACEAE							
Helwingia japonica (Thunb.) F. Dietr.	U42524	*H. japonica*	L11226	*H. japonica*	AF209596	*H. japonica*	AF130207
HYDRANGEACEAE							
Carpenteria californica Torr.	AJ235992*	*C. californica*	L11177	*C. californica*	AJ235426	*C. californica*	AF130217
Decumaria barbara L.	AJ235985*	*D. barbara*	AF206760	*D. barbara*	AJ236233*	*D. barbara*	AJ236252*
Fendlera rupicola Engelm. & A. Gray	AJ235986*	*F. rupicola*	AF206766	*F. rupicola*	AJ236234*	*F. rupicola*	AJ236253*
Hydrangea macrophylla (Thunb.) Ser.	U42781	*H. macrophylla*	L11187	*H. macrophylla*	AJ235497	*H. macrophylla*	AF130218
Philadelphus lewisii Pursh	AJ235984*	*P. lewisii*	L11198	*P. lewisii*	AJ236231*	*P. lewisii*	AJ236254*
HYDROLEACEAE							
Hydrolea ovata Nutt. ex Choisy	AJ236011*	*H. ovata*	L14293	*H. ovata*	AJ236184*	*H. ovata*	AF013999*
HYDROSTACHYACEAE							
Hydrostachys imbricata A. Juss.	AJ235983*	*H. multifida* A. Juss.	U17879	*H. imbricata*	AJ236230*	*H. multifida*	AF147712
ICACINACEAE							
Irvingbaileya sp.	AJ235999*	*Irvingbaileya* sp.	AF156733*	*Irvingbaileya* sp.	AJ236219*	*Irvingbaileya* sp.	AJ236255*
LAMIACEAE							
Clerodendrum chinense (Osb.) Mabb.	AJ236033*	*C. fragrans* Vent.	L11689	*C. chinense*	AJ236033	*C. chinense*	U78683
Laminum amplexicaule L.	L49287	*L. purpureum* L.	U75702	*L. amplexicaule*	AJ236165*	*Ajuga reptans* L.	L36391
Callicarpa dichotoma (Lour.) Koch	AJ236048*	*C. dichotoma*	L14393			*C. dichotoma*	L36395
LECYTHIDACEAE							
Couropita guianensis Aublet	AJ235993*	*C. guianensis*	Z80181	*C. guianensis*	AJ236224*	*C. guianensis*	AJ236256*
Gustavia superba (Kunth) O. Berg	AJ235994*	*G. poeppigiana* Berg	Z80175	*G. superba*	AJ236222*	*G. superba*	AJ236257*
Napoleonaea vogelii Hook. & Planchon	AF161009*	*N. vogelii*	Z80173	*N. vogelii*	AJ235540	*N. imperialis* Beauv.	AJ236258*
LENTIBULARIACEAE							
Pinguicula lutea Walter	AJ236043*	*P. caerulea* Walter	L01942				
Utricularia sp.	AJ236044*	*U. biflora* Roxb.	L12190				

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
LINNAEACEAE							
Abelia triflora R. Br.	AJ236004*	*Abelia* sp.	AF206727	*A. triflora*	AJ236209*	*A. grandiflora* L.	AF130193
LOASACEAE							
Loasa acuminata Wedd.	AJ235990*	*Caiophora laterita* Klotzsch	U17872	*L. acuminata*	AJ236228*	*L. acuminata*	AJ236259*
Eucnide bartonioides Zucc.	AJ235988*	*E. lobata* (J. D. Hook.) A. Gray	U17874	*E. bartonioides*	AJ236227*	*E. bartonioides*	AJ236260*
Mentzelia lindleyi Torrey & A. Gray	AJ235987*	*M. decapetala* (Pursh) Urb. & Gilg	U17877	*M. lindleyi*	AJ236235*	*M. lindleyi*	AJ236261*
Petalonyx nitidus Wats.	AJ235959*	*P. nitidus*	U96904	*P. nitidus*	AJ236232*	*P. nitidus*	AJ236262*
LOGANIACEAE							
Spigelia marilandica L.	AJ236024*	*S. marilandica*	L14007	*S. marilandica*	AF209679	*S. marilandica*	AF147713
MARCGRAVIACEAE							
Marcgravia rectiflora Triana & Planchon	Kron 1996	*M. rectiflora*	Z83148	*M. rectiflora*	AJ235529	*M. rectiflora*	AJ236263*
MELANOPHYLLACEAE							
Melanophylla alnifolia Baker	AJ236002*	*M. alnifolia*	U50254	*M. alnifolia*	AJ236212*	*M. alnifolia*	AJ236244*
MENYANTHACEAE							
Menyanthes trifoliata L.	AJ236009*	*M. trifoliata*	L14006	*M. trifoliata*	AJ235533	*M. trifoliata*	L39388
Nymphoides geminata (R. Br.) Kuntze	AJ236010*	*N. peltata* (Gmel.) Kuntze	X87392	*N. geminata*	AJ236204*	*N. indica* (L.) Kuntze	AF130181
MONTINIACEAE							
Montinia caryophyllacea Thunb.	U42808	*M. caryophyllacea*	L11194	*M. caryophyllacea*	AJ236187*	*M. caryophyllacea*	AF130178
MYOPORACEAE							
Myoporum mauritianum A. DC.	AJ236036*	*M. mauritianum*	L36445	*M. mauritianum*	AJ236166*	*M. mauritianum*	L36403
MYRSINACEAE							
Ardisia crenata Sims	AJ235997*	*A. crenata*	L12598	*Maesa myrsinoides* Lev.	AJ235525	*A. crenata*	AJ236264*
OLEACEAE							
Jasminum suavissimum Lindl.	AJ236035*	*J. suavissimum*	L01929	*J. polyanthum* Franchet	AJ235508	*J. mesnyi* Hance	AF130162
Olea europaea L.	L49289	*O. europaea*	AJ001766	*O. europaea*	AJ236163*	*O. europaea*	AF130163
OROBANCHACEAE							
Pedicularis lanceolata Michx.	U38317	*P. foliosa* L.	AF026836				
PAULOWNIACEAE							
Paulownia tomentosa (Thunb.) Steudel	AJ236039*	*P. tomentosa*	L36447	*P. tomentosa*	AJ236174*	*P. tomentosa*	L36406

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
PEDALIACEAE							
Proboscidea louisianica Thell.	AJ236040*	*P. louisianica*	L01946	*P. louisianica*	AJ236161*	*P. louisianica*	AJ236267*
Sesamum indicum L.	AJ236041*	*S. indicum*	L14408	*S. indicum*	AJ236176*	*S. indicum*	L36413
PHYLLONOMACEAE							
Phyllonoma laticuspis (Turcz.) Engl.	U42546	*P. laticuspis*	L11201	*P. laticuspis*	AJ236216*	*P. laticuspis*	AF130208
PITTOSPORACEAE							
Pittosporum japonicum C. Presl.	L28142	*P. japonicum*	L11202	*P. fairchildii* Cheesem.	AJ235561	*P. tobira* (Thunb.) Aiton	AF130201
Sollya heterophylla Lindl.	AJ236001*	*S. heterophylla*	U50262	*S. heterophylla*	AJ236214*	*S. heterophylla*	AJ236268*
PLANTAGINACEAE							
Callitriche heterophylla Pursh	AF107582*	*C. heterophylla*	L11681	*C. heterophylla*	AJ236164*	*C. hermaphroditica* L.	L36396
Plantago lanceolata L.	AJ236046*	*P. lanceolata*	L36454			*P. lanceolata*	L36408
POLEMONIACEAE							
Cobaea scandens Cav.	L24143	*C. scandens*	Z83143	*C. scandens*	AJ235440		
Gilia capitata Sims	L49295	*G. aggregata* (Pursh) Spreng.	Z83144	*G. capitata*	AJ236220*	*G. capitata*	AJ236269*
Phlox longifolia Nutt.	AJ235996*	*P. longifolia*	AF156732*	*P. longifolia*	AJ236221*	*P. drumondii* Hook.	AF130211
PRIMULACEAE							
Primula sp.	L49295	*Anagallis arvensis* L.	M88343	*A. tenella* (L.) L.	AJ235390	*A. arvensis*	AF130212
RORIDULACEAE							
		Roridula gorgonias Planchon	L01950	*R. gorgonias*	AJ236180*	*R. gorgonias*	AJ236270*
ROUSSEACEAE							
Roussea simplex Sm.	U42549	*R. simplex*	AJ235792	*R. simplex*	AJ235586		
RUBIACEAE							
Cephalanthus occidentalis L.	AJ236021*	*C. occidentalis*	X83629	*C. occidentalis*	AJ236190*	*C. occidentalis*	AJ236288
Erithalis fruticosa L.	AJ236028*	*E. fruticosa*	X83635	*E. fruticosa*	AJ236192*	*E. fruticosa*	AJ236295
Guettarda uruguensis Cham. & Schlecht.	AJ236029*	*G. uruguensis*	X83638	*G. uruguensis*	AJ236196*	*G. uruguensis*	AJ236297
Ixora coccinea L.	AJ236022*	*I. coccinea*	X83646	*Coffea arabica* L.	AJ235441	*I. coccinea*	AJ236299
Mitchella repens L.	U42802	*M. repens*	Z68805				
Pentas lanceolata (Forssk.) Deflers	AJ236027*	*P. lanceolata*	L13931	*P. lanceolata*	AJ236194*	*P. lanceolata*	AJ236304
Rogiera suffrutescens (Brand.) A. Borhidi	AJ26030*	*R. suffrutescens*	X83665	*R. suffrutescens*	AJ236191*	*R. suffrutescens*	AJ236308
SAPOTACEAE							
Manilkara zapota (L.) Royen	U43080	*M. zapota*	L01932	*M. zapota*	AJ235528		

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
SARRACENIACEAE							
Sarracenia purpurea L.	U42804	*S. flava* L.	L01952	*S. flava*	AJ235594	*S. purpurea*	AJ236271*
SCROPHULARIACEAE							
Antirhinum majus L.	AJ236047*	*A. majus*	L11688	*A. majus*	AJ235395	*A. majus*	L36392
Digitalis grandiflora Mill.	AJ236045*	*D. purpurea* L.	L01902	*D. grandiflora*	AJ236167*	*D. grandiflora*	L36399
Scrophularia californica Cham. & Schlect.	AJ236031*	*Scrophularia* sp.	L36449	*S. californica*	AJ236175*	*Scrophularia* sp.	L36411
Verbascum thapsus L.	AF161011*	*V. thapsus*	L36452	*V. thapsus*	AJ236177*	*V. thapsus*	L36417
Veronica anagallis-aquatica L.	AJ236055*	*V. persica* Poiret	L36453	*V. anagallis-aquatica*	AJ236169*	*V. persica*	L36419
SOLANACEAE							
Lycopersicon esculentum Mill.	AJ236017*	*L. esculentum*	L14403	*L. esculentum*	AJ236188*	*L. esculentum*	U08921
Nicotiana tabacum L.	AJ236016*	*N. tabacum*	Z00044	*N. tabacum*	AJ236183*	*N. tabacum*	L14953
Nolana humifusa (Gouan) I. M. Johnst.	AJ236017*	*N. spathulata* Ruiz & Pav.	U08616	*N. humifusa*	AF209638	*N. spathulata*	U08925
Petunia axillaris (Jussieu) Britton, Stern & Poggenb.	AJ236020*	*P. hybrida* (Hook. f.) Vilm.	X04976	*P. hybrida*	AJ236182*	*P. axillaris*	U08926
Schizanthus pinnatus Ruiz & Pavon	AF207016	*S. pinnatus*	U08619	*S. pinnatus*	AJ236185*	*S. pinnatus*	U08929
STILBACEAE							
Euthystachys abbreviata (E. Mey.) A. DC.	AJ236034*	*E. abbreviata*	Z29671	*E. abbreviata*	AJ236173*	*E. abbreviata*	AF147715
STYLIDIACEAE							
Stylidium graminifolium Sw.	AJ236011*	*S. graminifolium*	L18790	*S. graminifolium*	AJ236201*	*S. graminifolium*	AF130188
STYRACACEAE							
Styrax americana Lam.	U43296	*S. americana*	L12623	*S. japonicus* Siebold & Zucc.	AJ235615	*S. americana*	AF130215
SYMPLOCACEAE							
Symplocos paniculata Miq.	U43297	*S. paniculata*	L12624	*S. costata* Zoll.	AJ235617		
TERNSTROEMERIACEAE							
Eurya emarginata (Thunb.) Makino	AJ235995*	*E. japonica* Thunb.	Z80207	*E. japonica*	AF209585	*E. emarginata*	AJ236274*
THEACEAE							
Camellia japonica L.	U42815	*C. japonica*	L11219/ L12602	*C. japonica*	AF209553	*C. japonica*	AF130216
THEOPHRASTACEAE							
Clavija integrifolia Mart. & Miq.	AJ235998*	*C. eggersiana* Mez	L12608	*C. eggersiana*	AJ235437	*C. integrifolia*	AJ236275*

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
VAHLIACEAE							
Vahlia capensis Thunb.	U42813	*V. capensis*	L11208	*V. capensis*	AJ236217*	*V. capensis*	AJ236273*
VALERIANACEAE							
Valeriana officinalis L.	AJ236003*	*V. officinalis*	L13934	*V. officinalis*	AJ235637	*V. fauriei*	AF130192
VERBENACEAE							
Lantana camara L.	AJ236049*	*L. camara*	AF156736*			*L. horrida* Kunth.	AF130152
Phyla lanceolata (Michx.) Greene	AJ236051*		n.a.	*P. lanceolata*	AJ236162*	*P. incisa* Small	AF130153
Verbena bracteata Lag. & Rodr.	AJ236042*	*V. bonarensis* L.	L14412	*V. scabrido-glandulosa* Turrill	AF235639	*V. bracteata*	L36418
OUTGROUP							
AKANIACEAE							
Akania bidwillii (Hogg) Mabb.	L12568	*A. bidwilii*	L12568				
BUXACEAE							
Buxus sempervirens L.	L54065	*B. sempervirens*	X69729	*B. sempervirens*	AF092110	*B. sempervirens*	AJ236280*
CERATOPHYLLACEAE							
Ceratophyllum demersum L.	U42517	*C. demersum*	M77030	*C. demersum*	AJ235430	*C. demersum*	AF130232
CERCIDIPHYLLACEAE							
Cercidiphyllum japonicum Siebold & Zucc.	U42518	*C. japonicum*	L11673	*C. japonicum*	AF092112	*C. japonicum*	AF130224
CHENOPODIACEAE							
Spinacia oleracea L.	L24420	*S. oleracea*	J01443				
DILLENIACEAE							
Tetracera asiatica (Lour.) Hoogland	AJ235982*	*Dillenia indica* L.	L01903	*T. asiatica*	AJ235622	*T. asiatica*	AJ236277*
FAGACEAE							
Quercus rubra L.	AF132892*	*Q. rubra*	M58391	*Q. rubra*	AF20963	*Q. rubra*	AF130226
HAMAMELIDACEAE							
Liquidambar styracifolia L.	U42553	*L. styracifolia*	M58394				
ITEACEAE							
Itea virginica L.	U42545	*I. virginica*	L11188				
LIMNANTHACEAE							
Limnanthes douglasii R. Br.	Rodman	*L. douglasii*	L14700				

Appendix 1. Continued.

18S rDNA	GenBank	*rbc*L	GenBank	*atp*B	GenBank	*ndh*F	GenBank
LIRIODENDRACEAE							
Liriodendron chinense (Hemsl.) Sarg.	AJ235981*	*L. chinense*	L12654	*L. tulipifera* L.	AJ235522	*L. tulipifera*	AJ236278*
OXALIDACEAE							
Oxalis regnelli Miq.	AJ235980*	*O. dillenii* Jacq.	L01938	*O. regnelii*	AJ236236*	*O. regnelii*	AJ235278
PAEONIACEAE							
Paeonia suffructicosa Andrews	U42792	*P. tenuifolia* L.	L13687	*P. suffruticosa*	AF093389	*P. anomala* L.	AF130223
PAPAVERACEAE							
Dicentra eximia Torrey	L37908	*D. spectabilis* (L.) Lem.	L08761	*D. eximia*	L37927	*D. spectabilis*	AF130234
PHYTOLACCACEAE							
Phytolacca americana L.	U42793	*P. americana*	M62567	*P. dioica* L.	AF235558	*P. americana*	AF130229
PLUMBAGINACEAE							
Plumbago auriculata Lam.	U42795	*P. capensis* Thunb.	M77701	*P. zeylanica* L.	AF235565	*P. auriculata*	AJ236279*
RANUNCULACEAE							
Ramunculus sardous Crantz	L24092	*R. trichophyllus* Chaix	L08766				
RHAMNACEAE							
Rhamnus carthaticus L.	AJ235979	*R. carthaticus*	L13189	*R. carthaticus*	AF235579	*R. davurica* Pall.	AF130225
SABIACEAE							
Sabia swinhoei Hemsl.	L75840	*Sabia* sp.	L12662	*S. swinhoei*	AF093395*	*S. swinhoei*	AJ236276*
SAPINDACEAE							
Acer rubrum L.	U42494	*A. saccharum* L.	L01881	*A. saccharum*	AF035893*	*A. negundo* L.	AF130227
SAXIFRAGACEAE							
Saxifraga integrifolia Hook.	U42810	*S. integrifolia*	L01953				
Heuchera micrantha Douglas	X28139	*H. micrantha*	L01925	*H. micrantha*	AF039399	*H. micrantha*	AF130222
STAPHYLEACEAE							
Staphylea trifolia L.	AJ235978	*S. trifolia*	AJ238406	*S. trifoliata* Marsh.	AF235611	*S. emodii* Wall.	AF130228
TROCHODENDRACEAE							
Trochodendron aralioides Siebold & Zucc.	U42816	*T. aralioides*	L01958				
TROPAEOLACEAE							
Tropaeolum majus L.	L31796	*T. majus*	L14706	*T. tricolor* Lindl.	AF035917*	*T. majus*	AJ236281*

Appendix 2. Vouchers of sequences previously unpublished. Family names follow the Angiosperm Phylogeny Group (1998). Sequences marked with an asterisk are incomplete.

Species	Voucher/Source	Sequenced for
ACANTHACEAE		
Barleria prionitis L.	*Scotland 18* (OXF)	18S, *atp*B
Justicia americana (L.) Vahl	*Olmstead 90-011* (WTU)	18S, *atp*B
Thunbergia alata Sims	*Olmstead 93-045* (WTU)	18S
ACTINIDIACEAE		
Actinidia arguta (Siebold & Zucc.) Miq.	*Morgan s.n.* (WS)	*ndh*F
ADOXACEAE		
Sambucus ebulus L.	*Albach 11* (WS)	18S
Viburnum acerifolia L.	*Soltis 2561* (WS)	18S
ALSEUOSMIACEAE		
Alseuosmia macrophylla Cunn.	*Morgan 2141* (WS)	18S, *ndh*F, *atp*B
APOCYNACEAE		
Alstonia scholaris (L.) R. Br.	*Fanning 212* (FTG)	18S
Apocynum cannabinum L.	*Olmstead 256* (MICH)	18S
Nerium oleander L.	*Albach 3* (WS)	18S, *rbc*L, *atp*B*
AQUIFOLIACEAE		
Ilex opaca Soland.	*Soltis & Soltis 2527-2* (WS)	18S
ARALIACEAE		
Delarbrea micheana (F. v. Muell.) F. v. Muell.	*Plunkett 1366* (WS)	18S, *ndh*F, *atp*B
Panax quinquefolius L.	*Ware 10046* (WILLI)	18S, *atp*B
ASTERACEAE		
Barnadesia caryophylla (Vell.) S. F. Blake	*Jansen 911* (MICH)	18S
Gerbera jamesonii Bolus	*Albach 22* (WS)	18S, *atp*B
Helianthus annuus L.	*Albach 4* (WS)	18S, *atp*B
Tagetes sp.	*Nickrent 3061* (SIU)	*atp*B
Tragopogon dubius Scop.	*Soltis & Soltis 2472* (WS)	*atp*B*
BIGNONIACEAE		
Campsis radicans (L.) Bureau	*Course 0295a* (OSU)	18S*, *rbc*L, *atp*B
Catalpa bignonioides Walter	*Olmstead 92-099* (WTU)	18S
BORAGINACEAE		
Borago officinalis L.	*Olmstead 96-062* (WTU)	18S
Hydrophyllum fendleri (Gray) Heller	*Olmstead 957* (WTU)	18S
BUDDLEJACEAE		
Buddleja davidii Franch.	*Olmstead 88-007* (WTU)	18S
BRUNIACEAE		
Berzelia lanuginosa (L.) Brongn.	*Price s.n.* (IND)	*ndh*F, *atp*B*
BYBLIDACEAE		
Byblis gigantea Lindl.	*Palmengarten, Frankfurt*, same DNA as for 18S (Soltis et al., 1997a)	*atp*B
CALYCERACEAE		
Boopis graminea Phil.	*DeVore 1442* (OS)	18S, *atp*B
CAMPANULACEAE		
Codonopsis pilosula (Franch.) Nannf.	*Albach 10* (WS)	18S, *atp*B
CAPRIFOLIACEAE		
Symphoricarpus albus (L.) Blake	*Olmstead 89-006* (WTU)	*atp*B
CLETHRACEAE		
Clethra alnifolia L.	*Kron 1884s* (NCU)	*ndh*F
CONVOLVULACEAE		
Convolvulus arvensis L.	*Soltis 2581* (WS)	18S, *ndh*F, *atp*B
CORNACEAE		
Alangium chinense (Lour.) Harms	*U.S. Nat. Arb. 49003* (NA)	
Cornus officinalis Siebold & Zucc.	*Arnold Arb. 8156* (A)	
Davidia involucrata Baill.	*Seattle Arb. 265-72* (WTU)	18S, *ndh*F
Nyssa ogeche Marshall	*Xiang s.n.* (WS)	*ndh*F
DIAPENSIACEAE		
Galax urceolata (Poir.) Brummitt	*Johnson 97-010* (NCSU)	*ndh*F*, *atp*B*

Appendix 2. Continued.

Species	Voucher/Source	Sequenced for
DIPSACACEAE		
Scabiosa sp.	*Albach 39* (WS)	18S, *rbc*L, *atp*B*
DONATIACEAE		
Donatia sp.	*Morgan 2142* (WS)	18S, *ndh*F, *atp*B
EREMOSYNACEAE		
Eremosyne pectinata Endl.	*Annels & Hearn 4795* (UWA)	*ndh*F, *atp*B
ERICACEAE		
Arbutus unedo L.	*Seattle Arb. 652-60* (WTU)	18S, *ndh*F, *atp*B
Arctostaphylos uva-ursi (L.) Spreng	*Kron 164* (WFU)	*ndh*F*, *atp*B
ESACALLONIACEAE		
Escallonia rubra Ruiz & Pav.	*Albach s.n.* (WS)	*ndh*F*
FOUQUIERIACEAE		
Fouquieria splendens Engelm.	*Missouri Bot. Gard. 860162* (MO)	*ndh*F
GELSEMIACEAE		
Gelsemium sempervirens (L.) J. St.-Hil.	*Longwood Bot. Gard. 860395* (KEN)	18S, *atp*B
GENTIANACEAE		
Anthocleista grandiflora Gilg	same DNA as for *rbc*L (Olmstead et al., 1993)	18S, *ndh*F
Exacum affine Regel	*Matthaei Bot. Gard.*, same DNA as for *rbcL* and *ndhF* (Olmstead et al., 1992, 2000)	18S, *atp*B
GESNERIACEAE		
Cyrtandra hawaiensis Clarke	*Wagner 6753* (BISH)	18S, *atp*B
Drymonia urceolata Wiehler	*Smith 3416* (SRP)	18S
Nematanthus hirsutus (Mart.) Wiehler	*Marie Selby Bot. Gard.* (SEL), same DNA as for *rbcL* and *ndhF* (Olmstead & Reeves, 1995)	18S
Rhynchoglossum notonianum (Wall.) Burtt	*Smith 94-378* (SI)	18S, *atp*B
Streptocarpus holstii Engl.	*Matthaei Bot. Gard. 85003* (MICH)	18S
Titanotrichum oldhamii (Hemsl.) Soler.	*Smith 86-106* (SI)	18S, *atp*B
GOODENIACEAE		
Scaevola aemula R. Br.	*Albach 18* (WS)	18S
GRISELINIACEAE		
Griselinia littoralis (Raoul) Raoul	*Cameron s.n.* (AKU)	18S, *atp*B
HALESIACEAE		
Halesia diptera J. Ellis	*Ellis L49284* (NA)	*atp*B
HYDRANGEACEAE		
Carpenteria californica Torr.	*Soltis & Soltis 2478* (WS)	18S
Decumaria barbara L.	*Royal Bot. Gard. Kew 1969.50409* (K)	18S, *ndh*F, *atp*B
Fendlera rupicola Engelm. & Gray	*Abott 216* (UC)	18S, *ndh*F, *atp*B
Philadelphus lewisii Pursh.	*Soltis & Soltis 2411* (WS)	18S, *ndh*F, *atp*B
HYDROLEACEAE		
Hydrolea ovata Choisy	*Olmstead 89-009* (WTU)	18S, *atp*B
HYDROSTACHYACEAE		
Hydrostachys imbricata A. Juss.	*Schatz 3414* (MO)	18S, *atp*B*
ICACINACEAE		
Irvingbaileya sp.	*Plunkett 1510* (VCU)	18S, *rbc*L, *ndh*F*, *atp*B*
LAMIACEAE		
Clerodendrum chinense (Osb.) Mabb.	*Steane 88* (OXF)	18S
Callicarpa dichotoma (Lour.) Koch	*Olmstead 88-012* (WTU)	18S
Lamium amplexicaule L.	*Mort s.n.* (WS)	*atpB**
LECYTHIDACEAE		
Couropita guianensis Aublet	*Fairchild Trop. Gard. x-1-489* (FTG)	18S, *ndh*F, *atp*B
Gustavia superba (Kunth) O. Berg	*Fairchild Trop. Gard. 83189* (FTG)	18S, *ndh*F, *atp*B
Napoleonaea vogelii Hook. & Planch.	*Chase 329* (K)	18S, *ndh*F

Appendix 2. Continued.

Species	Voucher/Source	Sequenced for
LENTIBULARIACEAE		
Pinguicula lutea Walter	*Albach 1* (WS)	18S, *atp*B
Utricularia sp.	*Albach 6* (WS)	18S
LINNAEACEAE		
Abelia triflora R. Br.	*Seattle Arb. 2009-45* (WTU)	18S, *atp*B*
LOASACEAE		
Loasa acuminata Wedd.	*Albach 56* (WS)	18S, *ndh*F, *atp*B
Eucnide bartonioides Zucc.	*Albach 55* (WS)	18S, *ndh*F, *atp*B
Mentzelia lindleyi Torrey & A. Gray	*Albach s.n.* (WS)	18S, *ndh*F, *atp*B
Petalonyx nitidus Wats.	*Hufford 554* (DUL)	18S, *ndh*F, *atp*B
LOGANIACEAE		
Spigelia marilandica L.	*Beal Bot. Gard. XXB387* (MSC)	18S, *atp*B
MARCGRAVIACEAE		
Marcgravia rectiflora Triana & Planchon	*Chase 331* (K)	*ndh*F
MELANOPHYLLACEAE		
Melanophylla alnifolia Baker	*Schatz 3552* (MO)	18S, *ndh*F, *atp*B
MENYANTHACEAE		
Menyanthes trifoliata L.	*Peterson & Annable 3810* (WS)	18S
Nymphoides geminata (R. Br.) Kuntze	*Albach 61* (WS)	18S, *atp*B
MONTINIACEAE		
Montinia caryophyllacea Thunb.	*Williams 2833* (MO)	*atp*B
MYOPORACEAE		
Myoporum mauritanum A. DC.	*Royal Bot. Gard. Kew 1984-4220* (K)	18S, *atp*B
MYRSINACEAE		
Ardisia crenata Sims.	*Albach 5* (WS)	18S, *ndh*F
OLEACEAE		
Jasminum suavissimum Lindl.	*Matthaei Bot. Gard. 850259* (MICH)	18S
Olea europaea L.	*Albach s.n.* (WS)	*atp*B
PAULOWNIACEAE		
Paulownia tomentosa (Thunb.) Steudel	*Olmstead 88-008* (WTU)	18S, *atp*B
PEDALIACEAE		
Proboscidea lovisianica Thell.	*Morden 857* (IND)	18S, *ndh*F
Sesamum indicum L.	*Wagstaff 92-239* (WTU)	18S, *atp*B
PHYLLONOMACEAE		
Phyllonoma laticuspus (Turcz.) Engl.	*Morgan 2124* (WS)	*atp*B
PITTOSPORACEAE		
Sollya heterophylla Lindl.	*Plunkett 1361* (WS)	18S, *ndh*F, *atp*B
PLANTAGINACEAE		
Callitriche heterophylla Pursh	*Philbrick 2152* (CONN)	18S, *atp*B
Plantago lanceolata L.	*Albach 13* (WS)	18S
POLEMONIACEAE		
Gilia capitata Sims	*Johnson 92-15* (WS)	*ndh*F*, *atp*B
Phlox longifolia Nutt.	*Albach 20* (WS)	18S, *rbc*L, *atp*B
RORIDULACEAE		
Roridula gorgonias Planchon	*Goldblatt 5393* (MO)	*ndh*F, *atp*B
RUBIACEAE		
Cephalanthus occidentalis L.	*Forbes s.n.* (S)	18S, *atp*B
Erithalis fruticosa L.	*Meagher 990* (FTG)	18S, *atp*B
Guettarda uruguensis Cham. & Schlecht.	*Gillis 9575* (FTG)	18S, *atp*B
Ixora coccinea L.	*Bremer 2719* (CONN)	18S
Pentas lanceolata (Forssk.) Deflers	*Bremer 2702* (CONN)	18S, *atp*B
Rogiera suffrutescens (Brand.) A Borhidi	*Bremer 2712* (CONN)	18S, *atp*B
SARRACENIACEAE		
Sarracenia purpurea L.	*Morgan s.n.* (WS)	*ndh*F
SCROPHULARIACEAE		
Antirhinum majus L.	*Albach 57* (WS)	18S

Appendix 2. Continued.

Species	Voucher/Source	Sequenced for
Digitalis grandiflora Mill.	*Olmstead 92-015* (WTU)	18S, *atp*B
Scrophularia californica Cham. & Schlecht.	*Olmstead 93-89* (WTU)	18S, *atp*B
Verbascum thapsus L.	*Olmstead 92-113* (WTU)	18S, *atp*B
Veronica anagallis-aquatica L.	*Albach 37* (WS)	18S, *atp*B
SOLANACEAE		
Lycopersicon esculentum Mill.	*Albach 53* (WS)	18S, *atp*B
Nicotiana tabacum L.	*Albach 54* (WS)	18S
Nolana humifusa (Gouan) I. M. Johnst.	*Albach 19* (WS)	18S, *atp*B
Petunia axillaris (Jussieu) Britton, Stern & Poggenb.	*Olmstead S-60* (WTU)	18S, *atp*B
Schizanthus pinnatus Ruiz & Ravon	*Albach s.n.* (WS)	18S, *atp*B
STILBACEAE		
Euthystachys abbreviata (E. Mey.) A. DC.	*McDonald 92-288*, same DNA as for *rbc*L (Bremer et al., 1994) and *ndh*F (Wagstaff et al., 1998)	18S, *atp*B
STYLIDIACEAE		
Stylidium graminifolium Sw.	*M.E.D. 386* (UPS)	18S, *atp*B
TERNSTROEMIACEAE		
Eurya emarginata (Thunb.) Makino	*Schmidt, Merello & Sikes 1562* (MO)	18S, *ndh*F
THEACEAE		
Camellia japonica L.	*Nickrent 2929* (SIU)	*atp*B
THEOPHRASTACEAE		
Clavija intergrifolia Mart. & Miq.	*Fairchild Trop. Gard. 651364* (FTG)	18S, *ndh*F
VAHLIACEAE		
Vahlia capensis Thunb.	*Van Wyk 10-579* (PRU)	*ndh*F, *atp*B
VALERIANACEAE		
Valeriana officinalis L.	*Albach 2* (WS)	18S
VERBENACEAE		
Lantana camara L.	*Albach 12* (WS)	18S, *rbc*L
Phyla lanceolata (Michx.) Greene	*McCormac 4090* (OSU)	18S, *rbc*L, *atp*B
Verbena bracteata Lag. & Rodr.	*Olmstead 92-131* (WTU)	18S
OUTGROUP		
BUXACEAE		
Buxus sempervirens L.	*Hoot 921* (UWM)	*ndh*F
DILLENIACEAE		
Tetracera asiatica (Lour.) Hoogland	*Chase 123* (K)	18S, *ndh*F
MAGNOLIACEAE		
Liriodendron chinense (Hemsl.) Sarg.	*Qiu 28* (NCU)	18S
OXALIDACEAE		
Oxalis regnelli Miq.	*Soltis & Soltis 2548* (WS)	18S, *ndh*F*, *atp*B*
PLUMBAGINACEAE		
Plumbago auriculata Lam.	*Nickrent s.n.* (SIU)	*ndh*F*
RHAMNACEAE		
Rhamnus carthaticus L.	*Chase 100* (NCU)	18S
SABIACEAE		
Sabia swinhoei Hemsl.	*Wagner 6518* (HAST)	*ndh*F
STAPHYLEACEAE		
Staphylea trifolia L.	*Chase 16* (NCU)	18S
TROPAEOLACEAE		
Tropaeolum majus L.	*Chase 113* (NCU)	*ndh*F

Appendix 3. Insertions and deletions within the *ndh*F-data set; d.r.—direct repeat (determined by outgroup comparison using tree of combined analysis).

Char. #	Insertion/Deletion	bp	Taxa
1371	deletion	24	*Hydrolea*
1393	deletion	6	*Dicentra*
1396	deletion	9	*Hedera, Panax*
1417	deletion	6	*Anagallis, Ardisia*
1447	insertion	6	*Marcgravia, Donatia*
1453	deletion	9	*Titanotrichum, Aeschynanthus, Rhynchoglossum, Streptocarpus*
1456	deletion	9	Solanaceae
1456	deletion	3	*Impatiens*
1459	insertion	12	*Eremosyne*
1474	deletion	9	*Impatiens*
1477	deletion	6	*Callitriche*
1483	insertion	3	*Clavija*
1495	deletion	6	*Callitriche*
1498	insertion	9	all but *Liriodendron, Ceratophyllum, Buxus, Staphylea, Phytolacca, Dicentra*
1498	deletion	12	*Ardisia*
1501	deletion	9	*Helwingia, Phyllonoma, Vahlia, Griselinia,* Asterid I
1504	deletion	24	*Sabia*
1510	deletion	3	*Hydrostachys, Anagallis*
1510	deletion	9	*Borago*
1513	deletion	9	*Callitriche*
1515	insertion	9	*Pinguicula, Utricularia, Tetracera*
1524	deletion	6	*Anthocleista, Exacum, Pentas*
1524	deletion	48	*Ipomoea, Convolvulus*
1530	deletion	3	*Anthocleista, Exacum, Pentas, Gelsemium*
1534	deletion	3	*Veronica, Plantago, Digitalis, Callitriche*
1534	deletion	6	*Lobelia*
1537	deletion	6	*Veronica*
1537	deletion	18	*Callitriche*
1537	deletion	3	*Ilex, Helwingia, Phyllonoma*
1543	deletion	6	*Justicia, Barleria*
1543	deletion	21	*Plumbago*
1546	deletion	6	*Impatiens*
1547	insertion	3 d. r.	*Hydrostachys*
1560	deletion	9	*Sambucus, Viburnum*
1561	deletion	3	*Clerodendrum, Ajuga*
1563	insertion	6 d. r.	*Eremosyne, Gustavia, Hydrostachys*
1569	deletion	3	*Hydrostachys*
1579	deletion	9	*Paeonia*
1589	deletion	9	*Verbena*
1591	insertion	21 d.r.	*Schizanthus*
1591	deletion	9	*Scaevola*
1591	deletion	3	*Arctostaphylos, Arbutus*
1627	insertion	9	*Sabia*
1630	insertion	6	Lamiales s.l without Oleaceae, Gesneriaceae
1630	deletion	6	*Plumbago*
1637	deletion	6	*Pinguicula*
1646	deletion	3	*Cyrtandra*
1655	deletion	6	*Galax, Caiophora, Decumaria, Liriodendron, Tropaeolum, Cercidiphyllum*
1666	insertion	3 d.r.	*Hydrostachys*
1669	deletion	6	*Ajuga*
1678	deletion	30	*Ipomoea, Convolvulus*
1699	deletion	6	*Tetracera*
1783	deletion	3	*Ipomoea, Convolvulus*

Appendix 3. Continued.

Char. #	Insertion/Deletion	bp	Taxa
1786	insertion	9	*Plantago, Drymonia, Berzelia*
1786	insertion	6	*Anthocleista*
1795	deletion	9	*Montinia, Hydrolea*
1798	deletion	6	*Campanula, Codonopsis, Lobelia*
1804	deletion	9	*Jasminum, Olea*
1804	deletion	3	*Helianthus, Tragopogon, Tagetes*
1807	deletion	3	*Roridula*
1810	deletion	6	*Justicia, Barleria, Helianthus, Borago, Phacelia, Hydrophyllum*
1813	deletion	6	*Oxalis*
1861	insertion	6	*Tetracera, Plumbago*
1870	deletion	6	*Ipomoea, Convolvulus*
1882	insertion	18	*Donatia*
1993	insertion	6 d. r.	*Roridula*
2053	insertion	6 d. r.	*Gustavia, Delarbrea*
2074	insertion	6	*Rhamnus, Quercus, Acer*
2074	insertion	3 d. r.	*Actinidia, Roridula*

THE PHYLOGENETIC STATUS OF *POLYSCIAS* (ARALIACEAE) BASED ON NUCLEAR ITS SEQUENCE DATA[1]

Gregory M. Plunkett,[2] Porter P. Lowry II,[3] and Michele K. Burke[2]

Abstract

The circumscription of *Polyscias*, the second largest genus in Araliaceae (~130 species), has been broadened extensively in recent decades, but this circumscription has often been applied inconsistently, and no global treatment of the genus exists. To examine evolutionary relationships among the species of *Polyscias*, as well as the placement of this genus within Araliaceae, phylogenetic analyses were performed on a data set of 84 ITS sequences (from nuclear ribosomal DNA), including sequences from five of the formal sections of *Polyscias* and a representative sample of taxa from throughout Araliaceae and allied families. Results suggest that *Polyscias* is highly paraphyletic: members of nine other genera (*Gastonia*, *Cuphocarpus*, *Arthrophyllum*, *Reynoldsia*, *Tetraplasandra*, *Munroidendron*, *Meryta*, *Pseudopanax*, and *Schefflera* p.p.) are found nested within a broad "*Polyscias* sensu lato" clade. Despite a lack of support for the current definition of the genus, the phylogenetic relationships suggested by ITS data are remarkably consistent with geographic distributions. Three clades are clearly centered in the western Indian Ocean basin, and five others in the Pacific. These data further suggest that *Polyscias* sensu lato may have arisen in tropical Australasia, whence they may have migrated both east to the Pacific and west to the Indian Ocean, probably via long-distance dispersal after the breakup of Gondwanaland. Expanded studies, however, are required to address these biogeographic issues and to develop a revised generic-level taxonomy for *Polyscias* and its allies.

Key words: Araliaceae, biogeography, internal transcribed spacers, molecular phylogenetics, *Polyscias*.

Araliaceae are a family of mostly shrubs and small trees typically with solid stems, pinnately or palmately compound leaves, 5- to 8-merous flowers, and umbellate inflorescences often arranged in panicles or racemes. Although north temperate representatives such as *Aralia*, *Panax* (ginseng), and *Hedera* (English ivy) are widely recognized in many parts of the world, the majority of the ~50 genera and 1200–1400 species of Araliaceae are native to the tropics and temperate areas in the Southern Hemisphere. Relationships among araliad genera are poorly understood, and no recent studies have tested the late 19th- and early 20th-century classifications of the family (Bentham, 1867; Harms, 1894–97; Viguier, 1906, 1925). In fact, only two studies (Eyde & Tseng, 1971; Tseng & Hoo, 1982) in the last three decades have proposed any realignments of relationships among the genera of Araliaceae, and even these proposals have never been rigorously tested or applied.

As much as two thirds of the species diversity in Araliaceae may be found in just two genera, as currently circumscribed: *Schefflera* (~650–950 spp.) and *Polyscias* (~130 spp.). The sizes of both taxa have been increased substantially over the past several decades as they have been expanded to include many segregate groups (e.g., Frodin, 1975; Philipson, 1978, 1979). However, these and other taxonomic re-alignments in Araliaceae have been made largely without a phylogenetic perspective. To evaluate these changes and ultimately to develop a refined circumscription of both *Schefflera* and *Polyscias*, we have begun to collect data (molecular and non-molecular) from a broad sample of species representing the morphological and geographical spectrum of both genera, including members of

[1] The authors gratefully acknowledge J. Florence, D. Harder, D. Lorence, J.-N. Labat, and the herbaria and botanical gardens listed in Table 1 for supplying leaf tissue samples; J. Eibl for assistance in the laboratory; the staffs of IRD Nouméa and CSIRO (Atherton and Canberra) for assistance in New Caledonia and Australia, respectively; and two anonymous reviewers for valuable comments on the manuscript. Support for fieldwork was provided by grants from the National Geographic Society (5793–96 to GMP) and the National Science Foundation (DEB 9981641 to GMP and PPL, and DEB-9627072 to PPL as Co-PI); laboratory work was supported by a grant from the Thomas F. Jeffress and Kate Miller Jeffress Memorial Trust (J-506 to GMP).

[2] Department of Biology, Virginia Commonwealth University, Richmond, Virginia 23294-2012, U.S.A. gmplunke@vcu.edu.

[3] Missouri Botanical Garden, St. Louis, Missouri 63166-0299, U.S.A.; and Laboratoire de Phanérogamie, Muséum National d'Histoire Naturelle, 16 rue Buffon, 75005 Paris, France. lowry@mobot.org.

most segregate genera and infrageneric groups. Preliminary results from these investigations, along with a concurrent molecular survey of the entire family (Wen et al., 2001), suggest that both *Schefflera* and *Polyscias* may be paraphyletic or polyphyletic with regard to several other araliad genera. Thus, any attempt to re-evaluate evolutionary relationships within the family will require a simultaneous assessment of the taxonomic and phylogenetic status of *Schefflera* and *Polyscias*. Given the large number of species in each, we have chosen to assess their phylogenetic status separately, with the ultimate goal of developing a robust phylogeny and a new infrafamilial classification for the entire family (in conjunction with other workers). In the present study, we focus on relationships involving *Polyscias* and a number of closely related genera primarily from the Pacific and Indian Ocean basins.

Early definitions of *Polyscias* J. R. Forst. & G. Forst. included all araliads with imparipinnate leaves, panicles of umbels, articulated pedicels, and 5- to 8-merous flowers (Bentham, 1867; Seemann, 1868). This definition was maintained by Hutchinson (1967), but Stone (1965a, b) and later Bernardi (1971), Philipson (1978, 1979), and Lowry (1989) broadened *Polyscias* to include all Araliaceae with imparipinnate leaves and articulated pedicels, without regard to the number of flower parts. Following this redefinition, a number of genera were placed in synonymy under *Polyscias*, including *Bonnierella* R. Vig., *Botryopanax* Hutch., *Eupteron* Miq., *Gelibia* Hutch., *Kissodendron* Seem., *Nothopanax* Miq., *Palmervandenbroekia* Gibbs, *Sciadopanax* Seem., and *Tieghemopanax* R. Vig. (Bernardi, 1971; Lowry, 1989), four of which (*Eupteron, Gelibia, Kissodendron,* and *Palmervandenbroekia*) were formally recognized as sections within *Polyscias* (Philipson, 1978). However, other taxa (e.g., *Cuphocarpus*) have been maintained to date, despite their clear affinities to a broadly defined *Polyscias* (Bernardi, 1971, 1980). No comprehensive treatment of the genus currently exists, but based on published and unpublished information, we estimate that there are ~130 species of *Polyscias* (of which more than 20 remain to be described). The genus ranges from Africa, across the Indian Ocean basin (including Madagascar, and the Comoro and Mascarene archipelagos), through Malesia and Australia, to the south Pacific islands as far east as Tahiti.

As the first step in examining relationships involving *Polyscias* and its putative relatives, we derived DNA sequence data from internal transcribed spacers (ITS) of the nuclear ribosomal RNA genes (rDNA). Several earlier studies have demonstrated the phylogenetic utility of molecular data in helping to define relationships involving Araliaceae. For example, both *rbc*L and *mat*K sequences have been used to explore the troublesome relationship between Araliaceae and the closely related Apiaceae (= Umbelliferae) (Plunkett et al., 1996, 1997). These sequences have also provided some measure of resolution within each family, but application of protein-coding regions has not been as useful within Araliaceae as it has been in Apiaceae. This limitation is correlated with slower substitution rates of DNA sequences in Araliaceae, presumably due to differences in generation times among the mostly woody araliads compared to the primarily herbaceous umbellifers (see Plunkett & Lowry, 2001). At lower taxonomic levels, ITS sequence data have been successfully employed to examine relationships within and among several araliad genera, including *Aralia* and *Panax* (Wen & Zimmer, 1996) and *Pseudopanax* (Mitchell & Wagstaff, 1997). These studies indicate that ITS sequences sampled from throughout the family are easily aligned and that the evolutionary rate of ITS is appropriate for both intra- and intergeneric studies in Araliaceae. ITS sequence data have also been used successfully for phylogenetic studies in the closely related Apiaceae (Soltis & Kuzoff, 1993; Downie et al., 1998; Katz-Downie et al., 1999) as well as many other angiosperm groups (reviewed in Baldwin et al., 1995; Soltis & Soltis, 1998). Given the utility of ITS sequences, we have sampled taxa representing nearly all of the sections and segregate genera of *Polyscias* and from across the geographic range of the genus to assess its evolutionary relationships.

Materials and Methods

Total DNA was extracted from fresh or silica-gel dried leaf tissue of 71 species of Araliaceae (Table 1) using the CTAB method of Doyle and Doyle (1987) as modified by Soltis et al. (1991), or using the DNeasy Plant Mini kit (QIAGEN Inc.). For each taxon, the nucleotide sequence of the entire ITS region (including ITS1, ITS2, and the intervening 5.8S coding region) was derived by standard manual or automated sequencing protocols. In both cases, the ITS region was PCR-amplified using the forward primer ITS-5 and the reverse primer C26A [see White et al. (1990), Downie & Katz-Downie (1996), and Wen & Zimmer (1996) for all primer sequences]. Each 100 μL PCR reaction included 0.1–4 μL of unquantified template DNA, 2.5 units *Taq* DNA polymerase (Promega), 10 μL 10X *Taq*

buffer (supplied with the enzyme), 2.5 mM magnesium chloride, 0.8 mM total dNTPs, 5% DMSO, and a total of 0.5 μM primers. For PCR products used in automated sequencing, both primers were used in equimolar ratios to produce double-stranded amplicons. However, early trials indicated that asymmetric PCR products were superior templates for manual sequencing in *Polyscias*. In this approach, two PCR reactions were carried out on each sample, using the forward and reverse primers in ratios of 19:1 (producing mostly forward single-stranded amplicons), and 1:19 (producing mostly reverse single-stranded amplicons), respectively. In all cases, the PCR program included a pre-heating step at 94°C for 4 min., followed by 40 cycles of a 3-step program (94° for 30 sec. for DNA denaturation, 48° for 60 sec. for primer annealing, and 68° for 60 sec. for DNA extension), and completed with a final extension period (72°) for 15 min. Amplification products were cleaned using the procedure described by Morgan and Soltis (1993) or with the QIAquick PCR cleanup kit (QIAGEN Inc.). Manual sequences were obtained by Sequenase-mediated dideoxynucleotide reactions (U.S. Biochemical Corp.) using dGTP labeling mixes (or dITP mixes in regions prone to compressions) and ^{35}S-dATP. To obtain sequences of both (complementary) strands of the ITS region, four manual sequencing reactions had to be carried out for each taxon. On the forward amplicons, sequencing reactions were performed using reverse primers C26A and ITS-2; on the reverse amplicons, forward primers ITS-5 and N5.8S were used. Sequencing products were separated in 4% polyacrylamide gels, and autoradiograms were read manually. For sequences derived using automated procedures, only two reactions were required to obtain the sequence of both strands using ITS-5 and C26A as sequencing primers with the BigDye Terminator cycle sequencing reaction kit (PE Applied Biosystems) according to the manufacturer's instructions. Following the purification protocol recommended by the manufacturer, the sequencing products were separated by polyacrylamide gel electrophoresis on an ABI Prism 377 DNA Sequencer (PE Applied Biosystems). The resulting sequences were assembled and edited using the Sequencher (version 3.1) computer program (GeneCodes Corp.). Three taxa [*Polyscias murrayi*, *Gastonia cutispongia*, and *Arthrophyllum* "mackeei" (Lowry, ined.)] were sampled using both manual and automated protocols and thus serve as controls. With the exception of several bp (or regions) that could not be interpreted unambiguously, both approaches yielded identical sequences for each of these three taxa.

The 71 ITS sequences produced herein were combined with 13 previously published sequences (obtained from GenBank or directly from the authors) to yield a data matrix representing 84 species from three families (Table 1). Of these, four were included for outgroup comparisons based on the results of previous studies (e.g., Plunkett et al., 1996, 1997), including three species of Apiaceae and one Pittosporaceae. Of the 80 araliad sequences represented in the data set, 39 are from the genus *Polyscias* (as currently circumscribed), representing sections *Polyscias* (6 sequences), *Kissodendron* (1 sequence), *Gelibia* (1 sequence), and the *Tieghemopanax* group (17 sequences); the remaining species of *Polyscias* (14 sequences) are not presently assigned to any section or informal group (Table 2). Of the formally recognized infrageneric groups, only *Polyscias* sect. *Palmervandenbroekia* was not sampled because of difficulties obtaining material from members of this group, which is restricted to New Guinea. Forty-one additional sequences from Araliaceae (representing 25 genera) were included to test the monophyly and placement of *Polyscias*.

Initial alignments were performed using CLUSTAL X (Thompson et al., 1997) on a subset of ingroup and outgroup sequences, followed by visual refinements. After adding the remaining taxa, all sequences were then aligned manually. Gaps were treated as missing data. The data set was analyzed with PAUP* (vers. 4.0b2; D. Swofford, Smithsonian Inst.) using maximum parsimony (MP), maximum likelihood (ML), and distance (DIST) methods to infer phylogenetic relationships. Early parsimony searches yielded many thousands of most parsimonious trees, exhausting computer memory. Several large polytomies were identified as the source of this problem. To increase searching efficiency in the parsimony analyses, an initial search based on 100 replicates (with random addition, ACCTRAN optimization, and MULPARS in effect) was executed, saving no more than 100 trees per replicate. The strict consensus of this analysis was loaded as a topological constraint for an additional series of searches (1000 replicates, aborting each replicate after finding 1000 trees), saving only those trees not agreeing with the constraint. Because no additional topologies were found, the trees resulting from the initial search were loaded as starting trees with a MAXTREE limit of 10,000, and swapped to completion. To estimate confidence of individual clades within the MP trees, a bootstrap analysis (Felsenstein, 1985) was completed using PAUP*, and a decay analysis (Bremer, 1988; Donoghue et al., 1992) was performed using PAUP* in conjunc-

Table 1. Species, native range, source, and voucher information for the 84 ITS sequences used in the present study († = previously published sequences). Names and combinations indicated as "ined." will be published elsewhere; herbarium acronyms follow Holmgren et al. (1990).

Taxon	Native range	Source and voucher/Accession no.	GenBank accession
ARALIACEAE			
Polyscias			
Polyscias "abrahamiana" Lowry, ined.	Madagascar	Andasibe, Madagascar (*Labat 3064*, P)	AF229686
Polyscias albersiana Harms	Tanzania	W Usambara Mts., Tanzania (*Lowry 5153*, MO)	AF229687
Polyscias australiana (F. Muell) Philipson	Australia (Queensland), New Guinea	Near Bellenden Ker Park, northern Queensland, Australia (*Plunkett 1500*, MO)	AF229688
Polyscias balansae (Baill.) Harms	New Caledonia	Mé Ori, New Caledonia (*Lowry 4801*, MO)	AF229689
Polyscias baretiana Bernardi	Madagascar	Masoala Peninsula, Madagascar (*Aridy 281*, MO)	AF229690
Polyscias bellendenkerensis (F. M. Bailey) Philipson	Australia (Queensland)	Mt. Bartle Frere, northern Queensland, Australia (*Plunkett 1538*, MO)	AF229691
Polyscias "bracteata" (R. Vig.) Lowry, ined.	New Caledonia	Mt. Dzumac, New Caledonia (*Lowry 4663*, MO)	AF229692
Polyscias cissodendron (C. Moore & F. Muell.) Harms	New Caledonia	Mandjélia, New Caledonia (*Lowry 4749*, MO)	AF229693
Polyscias "crenata" (Pancher & Sebert) Lowry, ined.	New Caledonia	Baie de Tina, New Caledonia (*Lowry 4664*, MO)	AF229694
Polyscias cumingiana (C. Presl) Fern.-Vill.	Malesia; widely cult.	Po River, Between Ponérihouen and Houaïlou, New Caledonia (*Lowry 4788*, MO)	AF229695
Polyscias dioica (Vieill. ex Pancher & Sebert) Harms	New Caledonia	Mt. Dzumac, New Caledonia (*Lowry 4647*, MO)	AF229696
Polyscias cf. *dioica* (Vieill. ex Pancher & Sebert) Harms	New Caledonia	Roches Ouaïème, New Caledonia (*Lowry 4771*, MO)	AF229709
Polyscias "dzumacensis" Lowry, ined.	New Caledonia	Mt. Dzumac, New Caledonia (*Lowry 4650*, MO)	AF229697
Polyscias elegans (C. Moore & F. Muell.) Harms	Australia (Queensland), New Guinea	South Mission Beach, northern Queensland, Australia (*Plunkett 1495*, MO)	AF229698
Polyscias fulva (Hieron.) Harms	Tropical Africa	Bvumba Mts., Zimbabwe (*Lowry 4806*, MO)	AF229699
†*Polyscias fruticosa* (L.) Harms	Unknown, likely Melanesia; widely cult.	Mitchell & Wagstaff (1997)	U63191
†*Polyscias guilfoylei* (W. Bull) L. H. Bailey	Unknown, likely Melanesia; widely cult.	Wen et al. (2001)	AF242246
Polyscias cf. *guilfoylei* (W. Bull) L.H. Bailey	Unknown, likely Melanesia; widely cult.	Honolulu Botanical Garden (*Plunkett 1357*, WS)	AF229685
Polyscias "jaffrei" Lowry, ined.	New Caledonia	Kopéto Massif, New Caledonia (*Lowry 4740*, MO)	AF229700
Polyscias "lecardii" Lowry, ined.	New Caledonia	Mandjélia, New Caledonia (*Lowry 4754*, MO)	AF229701
Polyscias macguillivrayi (Seem.) Harms	Australia (Queensland), E Malesia, Solomon Islands, Micronesia	Cape Kimberly, northern Queensland, Australia (*Plunkett 1536*, MO)	AF229702
Polyscias mayottensis Lowry, O. Pascal & Labat	Comoro Islands	Saziley, Mayotte, Comoro Islands (*Labat 2935*, MO)	AF229703
Polyscias microbotrys (Baill.) Harms	New Caledonia	Mé Ori, New Caledonia (*Lowry 4802*, MO)	AF229704
Polyscias "modestei" Lowry, ined.	Madagascar	Ankirindro, Madagascar (*Schatz 3898*, MO)	AF229714
Polyscias mollis (Benth.) Harms	Australia (Queensland)	Bellenden Ker Park, northern Queensland, Australia (*Plunkett 1507*, MO)	AF229705

Table 1. Continued.

Taxon	Native range	Source and voucher/Accession no.	GenBank accession
Polyscias murrayi (F. Muell.) Harms	Australia (Queensland)	Bellenden Ker Park, northern Queensland, Australia (*Plunkett 1505*, MO)	AF229706
Polyscias "nothisii" Lowry, ined.	New Caledonia	Near Pouembout, New Caledonia (*Lowry 4739*, MO)	AF229707
Polyscias "orientalis" Lowry, ined.	Madagascar	Ankirindro, Madagascar (*Schatz 3925*, MO)	AF229708
Polyscias pancheri (Baill.) Harms	New Caledonia	Plaine des Lacs, New Caledonia (*Lowry 4693*, MO)	AF229710
Polyscias purpurea C. T. White	Australia (Queensland)	Licuala State Forest Park, northern Queensland, Australia (*Plunkett 1496*, MO)	AF229711
Polyscias "regalis" Bernardi ex Lowry, ined.	New Caledonia	Plateau de Dogny, New Caledonia (*Lowry 4720*, MO)	AF229712
Polyscias sambucifolia (Sieb. ex DC.) Harms	Australia	Australian National Botanical Garden (*Davies 1272*, CBG)	AF229713
Polyscias "scopoliae" (Baill.) Lowry, ined.	New Caledonia	Mt. Dzumac, New Caledonia (*Lowry 4638*, MO)	AF229715
Polyscias scutellaria (Burm. f.) Fosberg	Unknown, likely Melanesia; widely cult.	Flecker Botanical Garden, Queensland, Australia (*Plunkett 1491*, MO)	AF229716
Polyscias sessiliflora Marais	Reunion	Col de Bébour, Reunion (*Lowry 4981*, MO)	AF229717
Polyscias "subincisa" (R. Vig.) Lowry, ined.	New Caledonia	Mandjélia, New Caledonia (*Lowry 4750*, MO)	AF229718
Polyscias tahitiensis (Nadaud) Harms	Tahiti	Tahiti (*Florence 12911*, MO)	AF229719
Polyscias tennantii Bernardi	Madagascar	Andasibe, Madagascar (*Labat 3074*, P)	AF229720
Polyscias "veillonii" Lowry, ined.	New Caledonia	Mt. Ouin, New Caledonia (*Lowry 4658*, MO)	AF229721
Other Araliaceae			
Apiopetalum velutinum Baill.	New Caledonia	Mt. Mou, New Caledonia (*Lowry 4700*, MO)	AF229742
†*Aralia nudicaulis* L.		Wen & Zimmer (1996)	U41674
†*Aralia racemosa* L.		Wen & Zimmer (1996)	U41675
†*Aralia spinosa* L.		Wen & Zimmer (1996)	U66928
Arthrophyllum "mackeei" Lowry, ined.	New Caledonia	Haute Yaté, New Caledonia (*Lowry 4670*, MO)	AF229736
†*Arthrophyllum otopyrenum* (Baill.) Philipson (syn.: *A. angustatum*)	New Caledonia	Mitchell & Wagstaff (1997)	U63182
Astrotricha pterocarpa Benth.	Australia	Atherton Tablelands (Gillie's Lookout) northern Queensland, Australia (*Plunkett 1527*, MO)	AF229761
Cephalaralia cephalobotrys (F. Muell.) Harms	Australia (Queensland)	Near Herberton, northern Queensland, Australia (*Plunkett 1519*, MO)	AF229762
Cheirodendron faurei Hochr.	Hawaii	Na Pali Coast State Park, Kaua'i, Hawaii (*Harder 4070*, MO)	AF229763
Cheirodendron platyphyllum (Hook. & Arn.) Seem.	Hawaii	Na Pali Coast State Park, Kaua'i, Hawaii (*Harder 4072*, MO)	AF229764
Cuphocarpus aculeatus Decne. & Planch.	Madagascar	Sainte Luce, Madagascar (*Lowry 5013*, MO)	AF229737

Table 1. Continued.

Taxon	Native range	Source and voucher/Accession no.	GenBank accession
Cussonia spicata Thunb.	E Africa, Yemen, Comoro Islands	Missouri Botanical Garden (*Plunkett 1341*, WS)	AF229765
Delarbrea longicarpa R. Vig.	New Caledonia	Rivière Bleue Park, New Caledonia (*Lowry 4680*, MO)	AF229747
Gamblea ciliata C. B. Clarke subsp. *evodiaefolia* (Franch.) Shang, Lowry & Frodin	SW China, N Viet Nam	Fan Si Pan Mtn., Viet Nam (*Lowry 4856*, MO)	AF229766
Gastonia cutispongia Lam.	Reunion	Cult., Univ. de la Réunion, Reunion (*Lowry 4976*, MO)	AF229722
Gastonia rodriguensis Marais	Rodrigues Island	Royal Botanic Gardens Kew (acc. 662-86.06150)	AF229723
Mackinlaya confusa Hemsl.	Australia (Queensland)	Bellenden Ker Park, northern Queensland, Australia (*Plunkett 1512*, MO)	AF229743
Mackinlaya macrosciadia (F. Muell.) F. Muell.	Australia (Queensland)	Huntington Botanical Garden (*Plunkett 1365*, WS)	AF229744
Macropanax dispermus (Blume) O. Kuntze	India, Myanmar, S China, W Malesia	Bi Dùp, Viet Nam (*Lowry 4940*, MO)	AF229767
Meryta denhamii Seem.	New Caledonia	Mé Ori, New Caledonia (*Lowry 4793*, MO)	AF229725
Meryta "lecardii" (R. Vig.) Lowry, ined.	New Caledonia	Rivière Bleue Park, New Caledonia (*Lowry 4678*, MO)	AF229724
Munroidendron racemosum (Forbes) Sherff	Hawaii	Missouri Botanical Garden (*Plunkett 1342*, WS)	AF229738
Myodocarpus pinnatus Brongn. & Gris	New Caledonia	Plateau de Dogny, New Caledonia (*Lowry 4719*, MO)	AF229757
Osmoxylon geelvinkianum Becc.	New Guinea	Fletcher Botanical Garden, Queensland, Australia (*Plunkett 1489*, MO)	AF229727
Osmoxylon cf. *talaudense* Philipson	N Moluccas (Malesia)	National Tropical Botanical Garden, Hawaii (*Lorence 8158*, PTBG)	AF229726
†*Panax quinquefolius* L.		Wen & Zimmer (1996)	U41688
†*Pseudopanax arboreus* (Murr.) Philipson	New Zealand	Mitchell & Wagstaff (1997)	U63165
†*Pseudopanax chathamicus* Kirk	New Zealand (Chatham Island)	Mitchell & Wagstaff (1997)	U63166
Pseudosciadium balansae Baill.	New Caledonia	Mt. Mou, New Caledonia (*Lowry 4714*, MO)	AF229760
Reynoldsia sandwicensis A. Gray	Hawaii	Honolulu Botanical Garden, Hawaii (*Plunkett 1358*, WS)	AF229739
Schefflera candelabra Baill.	New Caledonia	Plateau de Dogny, New Caledonia (*Lowry 4726*, MO)	AF229728
Schefflera crassipes Baill.	New Caledonia	Mé Ori, New Caledonia (*Lowry 4797*, MO)	AF229729
Schefflera elegantissima (Veitch ex Masters) Lowry & Frodin	New Caledonia; widely cult.	Mt. Mou, New Caledonia (*Lowry 4715*, MO)	AF229730
Schefflera gabriellae Baill.	New Caledonia	Mt. Dzumac, New Caledonia (*Lowry 4648*, MO)	AF229731
Schefflera hypoleucoides Harms	S. China, Viet Nam	Fan Si Pan Mtn., Viet Nam (*Lowry 4920*, MO)	AF229732
Schefflera macrophylla (Dunn) R. Vig.	S. China, N Viet Nam	NW of Sa Pa, Viet Nam (*Lowry 4852*, MO)	AF229733
Schefflera pseudocandelabra R. Vig.	New Caledonia	Mé Ori, New Caledonia (*Lowry 4795*, MO)	AF229734
Sinopanax formosana (Hayata) H. L. Li	Taiwan	Jenai, Hsiang, Taiwan (*Lowry 4967*, MO)	AF229768

Table 1. Continued.

Taxon	Native range	Source and voucher/Accession no.	GenBank accession
Tetraplasandra hawaiiensis A. Gray	Hawaii	National Tropical Botanical Garden, Hawaii (*Lorence 8158*, PTBG)	AF229735
Tetraplasandra hawaiiensis A. Gray	Hawaii	Honolulu Botanical Garden, Hawaii (*Plunkett 1378*, WS)	AF229740
Tupidanthus calyptratus J. D. Hook. & Thoms.	India, Bangladesh, SE Asia	New York Botanical Garden (*Plunkett 1315*, WS)	AF229769
Outgroups			
PITTOSPORACEAE			
†*Pittosporum dallii* Cheesem.	New Zealand	Mitchell & Wagstaff (1997)	U63196
APIACEAE			
†*Daucus carota* L.	Eurasia, N Africa	Yokota et al. (1989)	X17534
†*Eryngium mexicanum* S. Watson	Mexico	Vargas et al. (1998)	AF031961
†*Sanicula canadensis* L.	E North America	Vargas et al. (1998)	AF031967

tion with the AutoDecay program (Eriksson, 1997). PAUP* and/or MacClade (Maddison & Maddison, 1992) were used to assess the levels of phylogenetic signal (skewness and PTP tests), homoplasy, transition to transversion ratio, and nucleotide composition. For the ML analysis, a heuristic search (with TBR branch swapping) was employed using empirical values for base-frequency rates and transition-to-transversion ratios; equal rates were assumed for variable sites, and a molecular clock was not enforced. In the distance analysis, heuristic searches were performed using both Tajima and Nei's (1984) model and the LogDet model of Lockhart et al. (1994; see also Gu & Li, 1996).

RESULTS

The length of the ITS region (including ITS1, 5.8S, and ITS2) for the 85 taxa included in the present study ranged from 613 bp to 628 bp. Alignment of the sequences required the addition of 48 gaps, mostly 1 to 3 bp long (although several gaps ranged from 4 to 11 bp). The total length of the ITS region following alignment was 705 bp. Of these, 309 bp (43.8%) were potentially parsimony-informative and 278 bp (39.4%) were invariable; the remaining 118 bp (16.7%) were variable in only a single taxon. The aligned length of the ITS1 region was 263 bp, that of ITS2 was 275 bp, and that of the 5.8S coding region was 165 bp. The transition to transversion ratio was 1.6:1, with a nucleotide composition of A = 19.3%; C = 36.9%; G = 25.5%; and T = 18.3%. The Tajima-Nei estimate of sequence divergence ranged from 0.16% [between *Polyscias balfouriana* and *P.* "jaffreyi" (Lowry, ined.)] to 45.2% (between *Daucus carota* and *Mackinlaya macrosciadia*) among all taxa in the sample (with the exception of the taxon designated "*Polyscias* c.f. *guilfoylei*," which was identical to the ITS sequence of *Polyscias cumingiana*). If the outgroup taxa are excluded, the maximum rate drops to 27.5% (between *Mackinlaya macrosciadia* and *Tupidanthus calyptratus*). The maximum rate among taxa sampled from within core Araliaceae is 18.1% (between *Osmoxylon geelvinkianum* and *Polyscias tahitiensis*), and 14.9% among taxa sampled from *Polyscias* (between *P. sambucifolius* and *P. tahitiensis*).

Cladistic analysis yielded greater than 10,000 most parsimonious (MP) trees of 1447 steps long. Excluding uninformative characters, the consistency index (CI) was 0.438 and the retention index (RI) was 0.683. The skewness test yielded a g_1 value of −0.451 and the PTP test a value of 0.004, both significant above the 95% confidence level.

Table 2. Placement of species examined from "*Polyscias* sensu lato" within the provisional groups recognized in the present study. No comprehensive taxonomic system currently exists for *Polyscias*, but placement in sections or segregate genera is provided for those species where available.

Informal group (based on molecular data)	Species	Traditional section or segregate genus
Arthrophyllum group	*Arthrophyllum* "mackeei" Lowry, ined.	n/a
	Arthrophyllum otopyrenum (Baill.) Philipson	n/a
	Polyscias australiana (F. Muell.) Philipson	Sect. *Kissodendron*
	Polyscias bellendenkerensis (F. M. Bailey) Philipson	Incertae sedis
	Polyscias mollis (Benth.) Harms	Sect. *Eupteron*
Gastonia group	*Cuphocarpus aculeatus* Decne. & Planch.	n/a
	Gastonia cutispongia Lam.	n/a
	Gastonia rodriguensis Marais	n/a
	Polyscias sessiliflora Marais	Incertae sedis
Meryta group	*Meryta denhamii* Seem.	n/a
	Meryta "lecardii" (R. Vig.) Lowry, ined.	n/a
	Polyscias murrayi (F. Muell.) Harms	Sect. *Eupteron*
	Pseudopanax arboreus (Murr.) Philipson	n/a
	Pseudopanax chathamicus Kirk	n/a
	Schefflera crassipes Baill.	n/a
	Schefflera elegantissima (Veitch ex Masters) Lowry & Frodin	n/a
	Schefflera gabriellae Baill.	n/a
Polyscias fulva group	*Polyscias albersiana* Harms	Incertae sedis
	Polyscias fulva (Hieron.) Harms	Incertae sedis
	Polyscias mayottensis Lowry, O. Pascal & Labat	Incertae sedis
Polyscias tennantii group	*Polyscias* "abrahamiana" Lowry, ined.	Incertae sedis
	Polyscias baretiana Bernardi	Incertae sedis
	Polyscias "modestei" Lowry, ined.	Incertae sedis
	Polyscias "orientalis" Lowry, ined.	Incertae sedis
	Polyscias tennantii Bernardi	Incertae sedis
Sect. *Polyscias* group	*Polyscias cumingiana* (C. Presl) Fern.-Vill.	Sect. *Polyscias*
	Polyscias fruticosa (L.) Harms	Sect. *Polyscias*
	Polyscias guilfoylei (W. Bull) L. H. Bailey	Sect. *Polyscias*
	Polyscias macguillivrayi (Seem.) Harms	Sect. *Polyscias*
	Polyscias purpurea C. T. White	Sect. *Eupteron*
	Polyscias scutellaria (Burm. f.) Fosberg	Sect. *Polyscias*
	Polyscias tahitiensis (Nadaud) Harms	Incertae sedis
Tetraplasandra group	*Munroidendron racemosum* (Forbes) Sherff	n/a
	Reynoldsia sandwicensis A. Gray	n/a
	Tetraplasandra hawaiiensis A. Gray	n/a
Tieghemopanax group	*Polyscias balansae* (Baill.) Harms	*Tieghemopanax*
	Polyscias "bracteata" (R. Vig.) Lowry, ined.	*Tieghemopanax*
	Polyscias cissodendron (C. Moore & F. Muell.) Harms	*Tieghemopanax*
	Polyscias "crenata" (Pancher & Sebert) Lowry, ined.	*Tieghemopanax*
	Polyscias dioica (Vieill. ex Pancher & Sebert) Harms	*Tieghemopanax*
	Polyscias "dzumancensis" Lowry, ined.	*Tieghemopanax*
	Polyscias elegans (C. Moore & F. Muell.) Harms	Sect. *Gelibia* or *Tieghemopanax*
	Polyscias "jaffreyi" Lowry, ined.	*Tieghemopanax*
	Polyscias "lecardii" Lowry, ined.	*Tieghemopanax*
	Polyscias microbotrys (Baill.) Harms	*Tieghemopanax*
	Polyscias "nothisii" Lowry, ined.	*Tieghemopanax*
	Polyscias pancheri (Baill.) Harms	*Tieghemopanax*
	Polyscias "regalis" Bernardi ex Lowry, ined.	*Tieghemopanax*
	Polyscias sambucifolius (Sieb. ex DC.) Harms	*Tieghemopanax*

Table 2. Continued.

Informal group (based on molecular data)	Species	Traditional section or segregate genus
	Polyscias "scopoliae" (Baill.) Lowry, ined.	*Tieghemopanax*
	Polyscias "subincisa" (R. Vig.) Lowry, ined.	*Tieghemopanax*
	Polyscias "veillonii" Lowry, ined.	*Tieghemopanax*

Results from the ML and NJ analyses (Figs. 2, 3) produced trees very similar to the MP trees. Visual comparison of the strict consensus of the MP trees to the ML and NJ trees confirms the monophyly of a group corresponding to "core Araliaceae," as defined by earlier studies (Plunkett, 1994; Plunkett et al., 1996, 1997). In each of the trees, *Astrotricha* and *Osmoxylon* form early diverging lineages at the base of the core Araliaceae clade. Relationships within core Araliaceae are not sensitive to outgroup selection; after exclusion of the divergent sequences from Apiaceae and Pittosporaceae, analyses yielded trees with few (DIST tree) or no (MP and ML trees) topological changes among core araliads. Inclusion of the outgroup did serve to confirm the distinctiveness of two small clades of araliads that fall outside of the core group. Recent studies have suggested that these two groups, comprising *Delarbrea*, *Pseudosciadium*, and *Myodocarpus*, and *Apiopetalum* and *Mackinlaya*, respectively, should probably be removed from core Araliaceae (see subsequent discussion).

All species of *Polyscias* are confined to a single large clade (see Figs. 1–3), although support for this clade is low [bootstrap (BS) = 38%; decay index (DI) = 2]. Within this "*Polyscias* sensu lato" clade, *Polyscias* (as currently defined) does not appear to be monophyletic. Rather, all three analyses concur in suggesting that the genus is paraphyletic with regard to nine other genera (viz., *Gastonia* Comm. ex Lam., *Cuphocarpus* Decne. & Planch., *Munroidendron* Sherff, *Tetraplasandra* A. Gray, *Reynoldsia* A. Gray, *Arthrophyllum* Blume, *Meryta* J. R. Forst. & G. Forst., *Pseudopanax* C. Koch, and *Schefflera* J. R. Forst. & G. Forst., p.p.). The three analyses also provide a very similar picture of relationships among the taxa within the *Polyscias* sensu lato clade, resolving eight identical (or nearly identical) species groupings, which we here provide with informal names for convenience (Table 2): (1) the "*Tieghemopanax* group" (BS = 67%; DI = 2; including all species sampled from the *Tieghemopanax* group of *Polyscias*, plus *P. elegans*, the sole member of the segregate genus *Gelibia*); (2) the "*Gastonia* group" (BS = 43%; DI = 1; including *Gastonia*, *Cuphocarpus*, and *Polyscias sessiliflora*); (3) the "*Polyscias fulva* group" (BS = 79%; DI = 3; *P. fulva*, *P. albersiana*, and *P. mayottensis*); (4) the "*Polyscias tennantii* group" [BS = 43%; DI = 1; *P. tennantii*, *P.* "abrahamiana" (Lowry, ined.), *P.* "modestei" (Lowry, ined.), *P. baretiana*, and *P.* "orientalis" (Lowry, ined.)]; (5) the "*Tetraplasandra* group" (BS = 99%; DI = 8; *Tetraplasandra*, *Reynoldsia*, and *Munroidendron*); (6) the "Sect. *Polyscias* group" (BS = 30%; DI = 1; all species sampled from *Polyscias* sect. *Polyscias*, plus *P. tahitiensis*); (7) the "*Arthrophyllum* group" (BS = 31%; DI = 1; both species sampled from *Arthrophyllum*, the single species sampled from *Polyscias* sect. *Kissodendron*, plus *P. bellendenkerensis* and *P. mollis*); and (8) the "*Meryta* group" (BS = 42%; DI = 1; including two species from *Meryta*, three from *Schefflera*, two from *Pseudopanax*, and possibly *Polyscias murrayi*). As with the broader *Polyscias* sensu lato clade, support for five of these eight smaller clades was weak (defined as less than 66%). In three of these cases, however, exclusion of a single basally branching taxon (*Polyscias mollis* from the *Arthrophyllum* clade, *P. purpurea* from the Sect. *Polyscias* clade, and *Cuphocarpus aculeatus* from the *Gastonia* clade) renders highly supported clades (with bootstrap values above 90%). The weak support and unstable placement of *Polyscias mollis* and *P. purpurea* (and to a lesser degree *P. murrayi* and *P. elegans*) are also reflected in a sensitivity to alternative alignments (data not shown) and to the method of analysis (cf. Figs. 1–3). In addition, relationships among the *Tetraplasandra* group and the three Indian Ocean clades (the *Gastonia*, *P. tennantii*, *P. fulva* groups), while identical in the MP and ML trees, differed somewhat in the DIST tree.

Discussion

Relationships in Araliaceae

The sampling strategy employed in the present analysis differs from that of other recent molecular studies of the family, but to the degree that there is sampling overlap, all recent molecular analyses yield a congruent picture of relationships at the family level. Two earlier studies, based on the chlo-

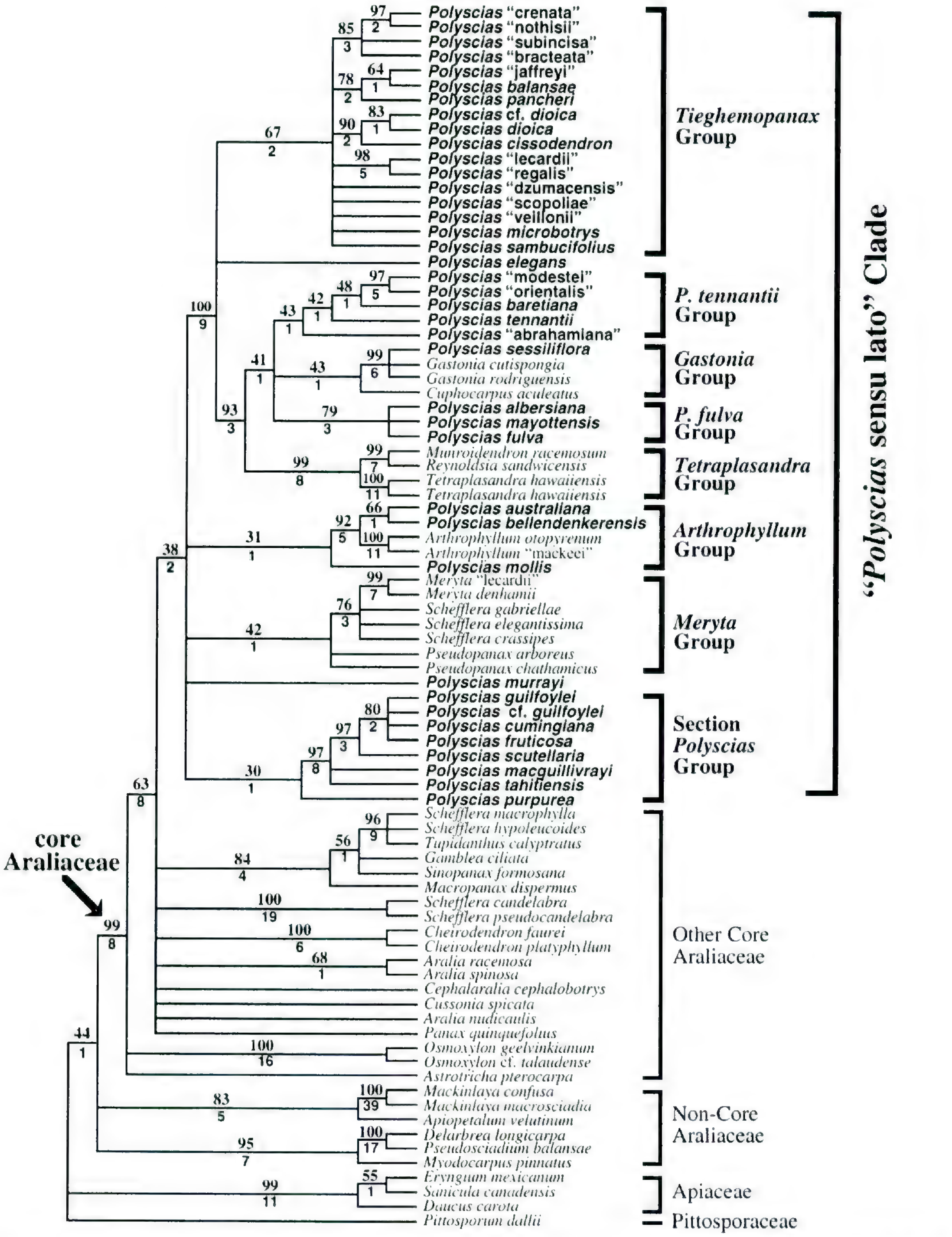


Figure 1. Strict consensus of 10,000 shortest trees resulting from the maximum parsimony (MP) analysis of 84 ITS sequences (representing 81 species of Araliaceae and four outgroup taxa); tree length = 1447 steps; consistency index = 0.438; retention index = 0.683. Species currently included within *Polyscias* are in boldface; names of major groups discussed in the text are provided next to brackets. Numbers above the branches are bootstrap percentages; those below the branches represent decay index values.

roplast genes *rbc*L and *mat*K (Plunkett et al., 1996, 1997), were designed to clarify the problematic relationship between Araliaceae and the closely related Apiaceae (which together comprise Apiales sensu Cronquist, 1988). These studies, which sampled broadly from throughout the order and several other closely related lineages (e.g., Pittosporaceae), helped to define two monophyletic "core" groups,

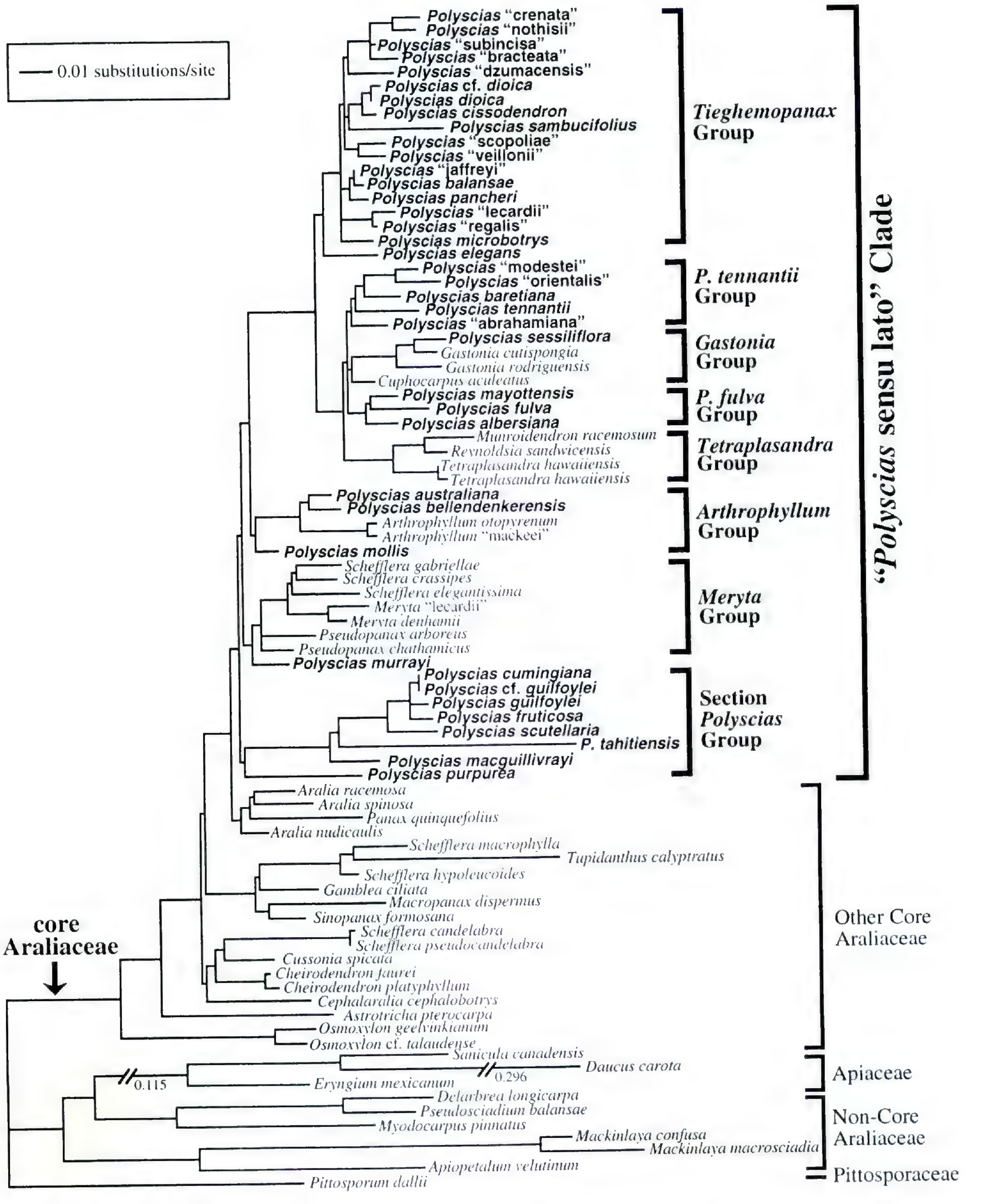


Figure 2. The phylogram resulting from maximum likelihood (ML) analysis of 84 ITS sequences from Araliaceae and outgroup taxa. Species currently included within *Polyscias* are in boldface. Branch lengths correspond to substitution rates; the scale of the two double-hatched branches is reduced to 25% (actual rate is provided below the branch).

which correspond largely to traditional taxonomic circumscriptions of the two families. Excluded from both core groups, however, were the araliads *Delarbrea* and *Mackinlaya,* as well as several genera from Apiaceae subfamily Hydrocotyloideae. Because the present study aims to examine the status and placement of *Polyscias* within core Araliaceae, we included only four species from outside the family. Nevertheless, the trees resulting from our analysis (Figs. 1–3) clearly resolve a core Araliaceae clade similar to that found in the cpDNA trees in excluding both *Delarbrea* and *Mackinlaya.* Moreover, in agreement with studies based on morphology, mating systems, and wood anatomy (e.g., Low-

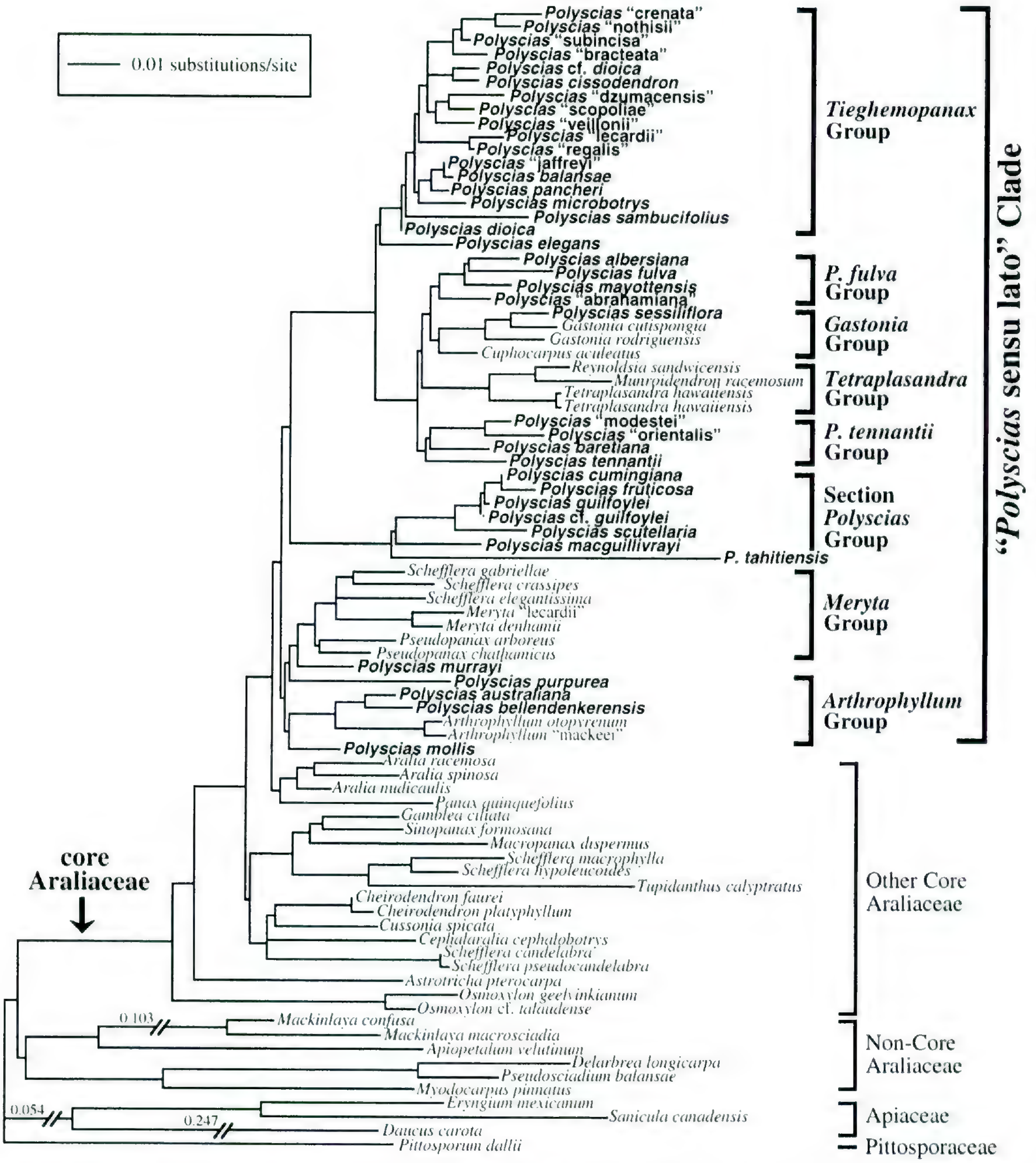


Figure 3. The phenogram resulting from distance (DIST) analysis of 84 ITS sequences from Araliaceae and outgroup taxa based on Tajima and Nei's (1984) model; the phenogram resulting from the analysis using the LogDet model (Lockhart et al., 1994) produced an identical topology (tree not shown). Species currently included within *Polyscias* are in boldface. Branch lengths correspond to substitution rates; the scale of the three double-hatched branches is reduced to 25% (actual rate is provided above the branch).

ry, 1986; Schlessman et al., 1990; Oskolski et al., 1997; Oskolski & Lowry, 2000), ITS data confirm the alliance of *Delarbrea* with *Pseudosciadium* and *Myodocarpus*, and of *Mackinlaya* with *Apiopetalum*. The MP tree (Fig. 1) suggests that *Delarbrea*, *Mackinlaya*, and their relatives form an unresolved polytomy with the core Araliaceae clade, but support for this is rather low (BS = 44%, DI = 1). In the ML tree, these clades are sister to the taxa from Apiaceae. This result mirrors the instability found in other studies regarding the placement of these "ancient araliads" (Plunkett et al., 1996, 1997; Plunkett & Lowry, 2001). In assessing relationships within core Araliaceae, results from the present study can also be compared to analyses using ITS sequences from a nearly comprehensive set of ar-

aliad genera (Wen et al., 2001). Again, where sampling overlap is sufficient, these studies provide a highly congruent picture of intergeneric relationships. For example, trees based on *rbc*L, *mat*K, and ITS data all suggest an alliance among *Tetraplasandra, Munroidendron, Reynoldsia, Gastonia,* and *Arthrophyllum,* and a close relationship among a group of mostly Asian genera (e.g., *Kalopanax, Trevesia,* and *Hedera*). Apart from such broad generalizations, however, a rigorous assessment of congruence among the various data sets will require additional sampling in order to build a complementary set of taxa for each marker.

THE STATUS OF *POLYSCIAS*

The trees based on ITS data suggest that the current circumscription of *Polyscias* is problematic. All species of *Polyscias* belong to a single clade within core Araliaceae, but this clade also includes all species sampled from eight additional araliad genera (*Cuphocarpus, Gastonia, Tetraplasandra, Munroidendron, Reynoldsia, Arthrophyllum, Meryta,* and *Pseudopanax*) and some (but not all) species of a ninth genus (*Schefflera*). Within this broad "*Polyscias* sensu lato" clade (see Figs. 1–3), there are eight major subclades, of which four include species from two or more genera. Applying the currently accepted taxonomy to the ITS trees, it is not possible to render a monophyletic *Polyscias.* The paraphyly of this genus is perhaps not surprising given its current, very broad circumscription. Neither of the two generally recognized diagnostic characters, imparipinnate leaves and articulated pedicels, can be applied unequivocally to separate *Polyscias* species from the other groups in the *Polyscias* sensu lato clade. For example, imparipinnate leaves are found in all members of the *Polyscias* sensu lato clade examined except the species of *Schefflera* and *Meryta,* although several *Polyscias* species have unifoliolate leaves [e.g., *P.* "scopoliae" (Lowry, ined.) from New Caledonia and an undescribed species from Madagascar]. Even in the simple-leaved *Meryta,* however, at least one species has strongly pinnatifid leaves, and several others have evident articulations along the midrib, suggesting that simple leaves may have been derived from pinnate leaves (by fusion of the leaflets into a single lamina). Pedicel articulation is less labile, but even with this character there are notable exceptions. For example, *Polyscias sessiliflora* from Reunion, *P. felicis* from the Comoro Islands, and *P. carolorum* (plus several undescribed species) from Madagascar all lack articulated pedicels, but have nevertheless been maintained in the genus (Bernardi, 1971, 1980; Marais, 1990). Conversely, all five species of *Cuphocarpus* have articulated pedicels as well as imparipinnate leaves, and yet the genus has been upheld as distinct on the basis of its unicarpellate gynoecium (Bernardi, 1971, 1980). Moreover, pedicel articulation is variable across the entire family. The pedicels of *Aralia, Panax, Hedera, Pseudopanax,* and *Cheirodendron* (inter alia) are all articulated, whereas those of many other genera are not (e.g., *Schefflera, Eleutherococcus, Tetrapanax, Trevesia,* and *Kalopanax*) (see Hutchinson, 1967). At the base of the core Araliaceae clade, *Astrotricha* possesses articulated pedicels but *Osmoxylon* lacks them, making it difficult to polarize this character. However, among those taxa now excluded from the core Araliaceae, *Delarbrea, Pseudosciadium, Myodocarpus,* and *Mackinlaya* all possess articulated pedicels and only *Apiopetalum* lacks them, suggesting that this feature may represent the ancestral character state (see Lowry et al., 2001; Plunkett, 2001). If this assumption is correct, articulations on the pedicels have been lost several times independently, both across Araliaceae and within the *Polyscias* sensu lato clade. In the attempt to identify other features that might be useful in circumscribing *Polyscias,* we examined inflorescence structure, stylar fusion, number of floral parts, degree of leaf division, features of the petiole base, breeding system, as well as habit. However, none of these consistently separate any of the seven genera from the species currently assigned to *Polyscias.* Thus, the paraphyly of *Polyscias* (as indicated by molecular data) does not contradict available morphological data.

The placement of three New Caledonian species of *Schefflera* (*S. gabriellae, S. elegantissima,* and *S. crassipes*) within the *Polyscias* sensu lato clade is difficult to explain on the basis of morphology. As discussed above, *Schefflera* (like *Polyscias*) currently has a very broad circumscription, including all araliads with palmately compound leaves but lacking pedicel articulations and prickles (Frodin, 1975, 1995). On the basis of floral vasculature patterns, Eyde and Tseng (1971) proposed that palmately leaved genera (such as *Schefflera*) and pinnately leaved groups (such as *Polyscias* and *Aralia*) represent fundamentally distinct lineages within Araliaceae. Their interpretation is supported in part by the present ITS analysis, although *Schefflera* itself appears to be polyphyletic (see also Wen et al., 2001). Moreover, support in the present study for the inclusion of the entire *Meryta* group (which includes these three *Schefflera* species) within the *Polyscias* sensu lato clade is rather low (BS = 38%, DI = 2). The ultimate placement of the *Schefflera*

species examined here can be assessed only after a more comprehensive sample from this genus has been assembled.

BIOGEOGRAPHIC RELATIONSHIPS

In contrast to the discordance between the currently accepted taxonomy and the trees based on ITS data, the topologies depicted in Figures 1–3 correspond remarkably well to geographic distributions. For example, the *Gastonia* group is clearly centered in the Indian Ocean: *Cuphocarpus* is endemic to Madagascar (Bernardi, 1971), *Polyscias sessiliflora* occurs on Reunion Island (Marais, 1990), and *Gastonia* is distributed from the Comoro Islands, Madagascar, the Seychelles and Mascarene Islands across to Malesia and the Solomon Islands in the western Pacific (Philipson, 1970). Two other clades are also restricted to the western Indian Ocean basin. The *P. fulva* group comprises *P. fulva* (from tropical Africa), *P. albersiana* (endemic to Tanzania), and *P. mayottensis* (from Mayotte in the Comoro Islands). Three species from Madagascar—*P. baehniana* (Bernardi) Bernardi, *P. boivinii* (Seem.) Bernardi, and *P. floccosa* (Drake) Bernardi—may also belong to the *P. fulva* group (cf. Lowry et al., 1999), but could not be sampled for the present study. The *P. tennantii* group (*P. tennantii*, *P.* "abrahamiana," *P.* "modestei," *P. baretiana*, and *P.* "orientalis") is entirely endemic to Madagascar. These groups together form a single clade with the *Gastonia* group.

Among the other clades, several are clearly centered in the western Pacific Ocean. The *Tieghemopanax* group is concentrated in New Caledonia, with five species occurring elsewhere in the southwest Pacific, two in Fiji (Smith, 1985), two in Vanuatu (Lowry, 1989), and one in Queensland (*P. sambucifolius*, sampled herein). Unfortunately, we were unable to obtain DNA from any of the species native to Fiji or Vanuatu.

The *Arthrophyllum* group reveals a similar biogeographic relationship between taxa studied from New Caledonia (two species of *Arthrophyllum*) and Queensland (*Polyscias australiana*, *P. bellendenkerensis*, and *P. mollis*), but this clade probably extends west into Malesia, where a number of unsampled species of *Arthrophyllum* occur (cf. Philipson, 1979). The native distribution of species in the Sect. *Polyscias* group extends from eastern Malesia and northeastern Australia through Melanesia and Micronesia to Polynesia. Several widely cultivated taxa, including *P. cumingiana*, *P. fruticosa*, *P. guilfoylei*, and *P. scutellaria*, are presumed to have arisen in Malesia (Philipson, 1979; Lowry, 1989). *Polyscias tahitiensis* has never been formally assigned to a section, but its placement in the Sect. *Polyscias* group is consistent with both geographic distribution and morphological characters for the group.

Lastly, the *Tetraplasandra* group is centered in Hawaii (where *Tetraplasandra* and *Munroidendron* are endemic), but *Reynoldsia* also occurs as far south and west as French Polynesia and Samoa (Philipson, 1970; Lowry, 1990). Unlike the three Indian Ocean groups (which form a single large clade), the Pacific clades are not monophyletic. In fact, ITS sequence data suggest that the *Tetraplasandra* group (from the eastern extreme of the range) is more closely related to the Indian Ocean groups (from the western extreme) than to any of the other Pacific groups.

Although our current sample of *Polyscias* is broad, it is not comprehensive. We have been able to include only three species native to Malesia (including *P. cumingiana*, *P. australiana*, and *P. elegans*, the latter two also present in Australia) and none from Sect. *Palmervandenbroekia*. Given this limitation, it would currently be imprudent to subject these data to formal biogeographic analysis. However, a preliminary assessment of biogeographic relationships within *Polyscias* sensu lato was made by mapping geographic distributions along the ITS cladogram (Fig. 2) using the character-transformation capabilities of MacClade. In this exercise, the ML topology was used because it is fully resolved; it should be remembered, however, that bootstrap and decay index support for several of the *Polyscias* clades is rather weak in the MP trees (Fig. 1), and thus data from other markers will be required to confirm the following biogeographic hypotheses. The area cladogram (Fig. 4) that resulted from this exercise suggests that the ancestral range for *Polyscias* and its allied genera was tropical Australia. Although *Polyscias* is rather species-poor in Australia (with 11 native species present; Lowry, unpublished data), it is quite diverse in nearby eastern Malesia, where 20 native species occur (cf. Philipson, 1979). Tropical Australia and Malesia have strong floristic affinities in many groups (Webb & Tracey, 1981; Hartley, 1986; but see Adam, 1992), and among Araliaceae more than half of the species in Australia either extend into Malesia or have close relatives there (Lowry, unpublished data; see also Philipson, 1979; Hartley, 1986). It is thus quite possible that tropical Australasia as a whole, and in particular Malesia, may represent the center of origin for the *Polyscias* sensu lato clade.

In our sample, Australian (i.e., tropical Australasian) taxa represent basally branching lineages in four of the major clades of *Polyscias* sensu lato

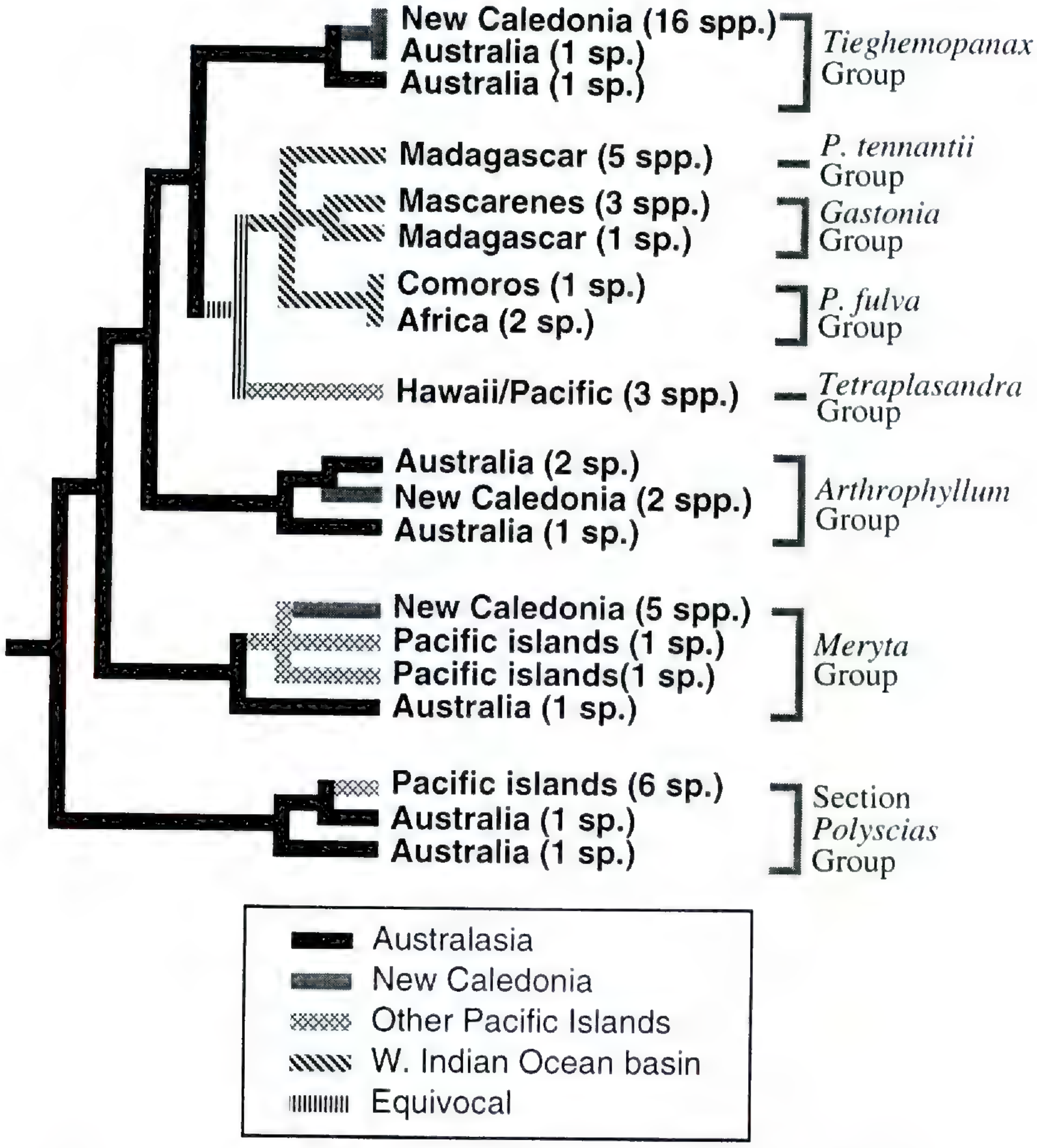


Figure 4. Simplified portion of the ML topology representing the "*Polyscias* sensu lato" clade and showing broad patterns of biogeographic relationships. Presented as an area cladogram, the tree was constructed by coding broad-scale geographic distributions (either Australasia, New Caledonia, other Pacific islands, or western Indian Ocean basin) of each taxon as character states, and then tracing the character-state transformations along the ML topology using MacClade.

(viz., the *Tieghemopanax* group, the *Arthrophyllum* group, the *Meryta* group, and the Sect. *Polyscias* group; Figs. 1–3). The phylogenetic alliance of the *Tetraplasandra* group to the three Indian Ocean clades (the *Gastonia* group, the *P. fulva* group, and the *P. tennantii* group) at first appears surprising, given that they are centered in the eastern and western extremes of the distributional range, respectively. The origin of the combined *Tetraplasandra*–Indian Ocean clade is equivocal (Fig. 4), but a single Australasian origin might explain the alliance of such geographically distant lineages. Such an interpretation is reinforced by comparing the distributions of successive sister groups of the *Tetraplasandra*–Indian Ocean clade (viz., the *Tieghemopanax* group and the *Arthrophyllum* group), both of which appear to be Australasian in origin (Fig. 4).

Polyscias and its allies exhibit a paleotropical distribution, and given the likely Cretaceous origins of Araliaceae (Raven & Axelrod, 1972, 1974; cf. also Lowry, 1986; Plunkett & Lowry, 2001), this distribution pattern could be explained either by ancient vicariance (viz., the Gondwanan breakup) or by more recent (post-tectonic) dispersals. If vicariance were responsible for the overall distribution of *Polyscias* throughout the Gondwanan landmasses, an early ancestor of *Polyscias* must have been widespread, and diversification therefore followed the tectonic events. In this case, each major clade would correspond to (or at least be concentrated in) a single geographic region. Alternatively, if *Polyscias* were narrowly distributed before the Gondwanan breakup, then long-distance dispersal must be invoked to explain the current distribution. Under this scenario, the ancestral geographic region would have served as the source for subsequent dispersal events, and taxa from the ancestral

area would be represented within all or many of the major clades of the phylogenetic tree. Mindful of the sampling limitations in the present study, our results favor the latter scenario, with taxa from Australasia (the hypothesized area of origin) found among the basally branching lineages of nearly all the major clades.

New Caledonia presents an interesting example. This island separated from Australia about 74 mya (Kroenke, 1996) during one of the greatest periods of angiosperm diversification, and this vicariance is responsible for the presence of many well known relicts from a number of ancient vascular plant lineages (Lowry, 1998). At the same time, the island lies just 1200 km off the coast of Queensland, well within the conceivable range for long-distance dispersal, especially for groups with fleshy fruits apparently well adapted for endozoochory (such as *Polyscias*). Again, the patterns illustrated in our ITS cladograms appear to support a dispersalist explanation for the presence of *Polyscias* sensu lato in New Caledonia, with at least three independent origins of New Caledonian lineages from Australasian ancestors (i.e., in the *Tieghemopanax* group, the *Arthrophyllum* group, and the *Meryta* group). This interpretation assumes, however, that diversification in these three clades occurred after the separation of New Caledonia from Australia, which cannot be verified without fossil evidence or additional molecular data (to test molecular clock assumptions).

By contrast, for relationships between Australasian and Pacific island taxa (in the Sect. *Polyscias* group, the *Meryta* group, and the *Tetraplasandra* group; Table 2), we can reasonably assume that current distributions represent the results of independent long-distance dispersal events given the comparatively recent origin of Hawaii and the other volcanic Polynesian islands. Such dispersalist scenarios differ markedly from largely vicariance explanations previously invoked for explaining the distribution of many of the ancestral lineages of Apiales (e.g., *Delarbrea, Myodocarpus, Apiopetalum* in New Caledonia; see Lowry, 1989; Schlessman et al., 1990, 2001; Plunkett et al., 1996, 1997; Plunkett & Lowry, 2001).

TAXONOMIC CONSIDERATIONS

The current study suggests that the phylogenetic history of *Polyscias* is complex. Clearly, additional sampling from the ~130 species of *Polyscias* (particularly from under-represented groups in Malesia) would be of value in assessing the status of this genus. Nevertheless, it is difficult to envision the maintenance of *Polyscias* (as currently circumscribed) given its evident paraphyly. Ultimately, *Polyscias* needs to be either partitioned into a series of smaller segregate genera or greatly enlarged (by submerging within it many currently recognized taxa). We herein advocate strongly the former course, for two reasons. First, the enlargement of *Polyscias* would result, essentially, in a "supergenus" equivalent to many tribes (or even subfamilies) in earlier taxonomic treatments. While this accomplishes monophyly at the generic level, it relegates the phylogenetic difficulties inherent in these groups to a lower taxonomic level. Such a solution would obscure rather than enlighten phylogenetic relationships in Araliaceae. Second, results from the current study suggest that a judiciously applied "splitting" approach need not lead to the recognition of numerous small genera. To use an example based solely on the results of the current study, each of six major clades (viz., *Tieghemopanax*, Sect. *Polyscias*, the *Arthrophyllum* group, the *Meryta* group, the *Tetraplasandra* group, and a combined Indian Ocean group) could be recognized as a distinct genus. This approach would circumscribe monophyletic groups while avoiding the problem of a single massive genus, and it would reduce the total number of recognized genera from nine to six. Some difficulties may, however, be encountered in identifying synapomorphies that can be used to define and recognize each group. Nevertheless, although we consider formal taxonomic changes in *Polyscias* premature at this time, we advocate using the names applied to the groups in Figures 1–3 (and listed in Table 2) to denote informal taxa until relationships within *Polyscias* can be more fully resolved and tested. This approach allows easy reference to phylogenetically and biogeographically meaningful groups (i.e., clades or lineages) without adding yet another layer of confusion to an already complicated taxonomic history. As a stable picture of relationships emerges, well-supported groups can then be defined and formally assigned generic names.

Literature Cited

Adam, P. 1992. Australian Rainforests. Clarendon Press, Oxford.

Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell & M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Gard. 82: 247–277.

Bentham, G. 1867. Araliaceae. Pp. 931–947 *in* G. Bentham & J. D. Hooker (editors), Genera Plantarum, Vol. 1. A. Black, W. Pamplin, Lovell Reeve, London.

Bernardi, L. 1971. Araliacearum Madagascariae et Comores propositum. 2. Revisio et taxa nova Polysciadum. Candollea 26: 12–89.

———. 1980. Synopsis Araliacearum Madagascariae et Comorarum Insularum (auxilio methodi "Ferulago"). Candollea 35: 117–132.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

Cronquist, A. 1988. The Evolution and Classification of Flowering Plants, 2nd ed. New York Botanical Garden, Bronx, New York.

Donoghue, M. J., R. G. Olmstead, J. F. Smith & J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann. Missouri Bot. Gard. 79: 333–345.

Downie, S. R. & D. S. Katz-Downie. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Amer. J. Bot. 83: 234–251.

———, S. Ramanath, D. S. Katz-Downie & E. Llanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpo*C1 intron sequences. Amer. J. Bot. 85: 563–591.

Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Eriksson, T. 1997. AutoDecay version 2.9.9 (hypercard-stack computer program distributed by the author). Botaniska Institutionem, Stockholm University, Stockholm, Sweden.

Eyde, R. H. & C. C. Tseng. 1971. What is the primitive flora structure of Araliaceae? J. Arnold Arbor. 52: 205–239.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Frodin, D. G. 1975. Studies in *Schefflera* (Araliaceae): The *Cephaloschefflera* complex. J. Arnold Arbor. 56: 427–448.

———. 1995. Neotropical montane Araliaceae: An overview. Pp. 421–431 *in* S. P. Churchill et al. (editors), Biodiversity and Conservation of Neotropical Montane Forests. New York Botanical Garden, Bronx, New York.

Gu, X. & W.-H. Li. 1996. Bias-corrected paralinear and LogDet distances and tests of molecular clocks and phylogenies under nonstationary nucleotide frequencies. Molec. Biol. Evol. 13: 1375–1383.

Harms, H. 1894–1897. Araliaceae. Pp. 1–62 *in* A. Engler & K. Prantl, Die natürlichen Pflanzenfamilien III, Vol. 8. W. Engelmann, Leipzig.

Hartley, T. G. 1986. Floristic relationships of the rainforest flora of New Guinea. Telopea 2: 619–630.

Holmgren, P. K., N. H. Holmgren & L. C. Barnett. 1990. Index Herbariorum. New York Botanical Garden, Bronx, New York.

Hutchinson, J. 1967. The Genera of Flowering Plants, Vol. 2. Oxford Univ. Press, London.

Katz-Downie, D. S., C. M. Valiejo-Roman, E. I. Terentieva, A. V. Troitsky, M. G. Pimenov, B. Lee & S. R. Downie. 1999. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: Additional information from nuclear ribosomal DNA ITS sequences. Pl. Syst. Evol. 216: 167–195.

Kroenke, L. W. 1996. Plate tectonic development of the western and southwestern Pacific: Mesozoic to the present. Pp. 19–34 *in* A. Keast & S. E. Miller (editors), The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes. SPB Academic Publ., Amsterdam.

Lowry, P. P., II. 1986. A systematic study of *Delarbrea* Vieill. (Araliaceae). Allertonia 4: 169–202.

———. 1989. A revision of Araliaceae from Vanuatu. Bull. Mus. Natl. Hist. Nat., Paris, ser. 4, sect. B, Adansonia 11: 117–155.

———. 1990. Araliaceae. Pp. 224–237 *in* W. L. Wagner, D. L. Herbst & S. H. Sohmer (editors), Manual of the Flowering Plants of Hawai'i, Vol. 1. Univ. Hawai'i Press, and Bishop Museum Press, Honolulu.

———. 1998. Diversity, endemism, and extinction in the flora of New Caledonia. Pp. 181–206 *in* C.-I Peng & P. P. Lowry II (editors), Proc. Intl. Symposium on Rare, Threatened, and Endangered Floras of Asia and the Pacific. Academia Sinica, Taipei, Taiwan.

———, O. Pascal & J.-N. Labat. 1999. A new species of *Polyscias* (Araliaceae) from Mayotte, Comoro Islands. Adansonia, sér. 3, 21: 67–73.

———, G. M. Plunkett & A. A. Oskolski. 2001. Early lineages in Apiales: Insights from morphology, wood anatomy and molecular data. Edinburgh J. Bot. 58 (in press).

Lockhart, P. J., M. A. Steel, M. D. Hendy & D. Penny. 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. Molec. Biol. Evol. 11: 605–612.

Maddison, W. P. & D. R. Maddison. 1992. MacClade: Analysis of Phylogeny and Character Evolution, Version 3.01. Sinauer, Sunderland, Massachusetts.

Marais, W. 1990. Araliaceae. Fl. Mascareignes 106: 1–20.

Mitchell, A. D. & S. J. Wagstaff. 1997. Phylogenetic relationships of *Pseudopanax* species (Araliaceae) inferred from parsimony analysis of rDNA sequence data and morphology. Pl. Syst. Evol. 208: 121–138.

Morgan, D. R. & D. E. Soltis. 1993. Phylogenetic relationships among members of Saxifragaceae sensu lato based on *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 631–660.

Oskolski, A. A. & P. P. Lowry II. 2000. Systematic wood anatomy of *Mackinlaya* and *Apiopetalum* (Araliaceae). Ann. Missouri Bot. Gard. 87: 171–182.

———, ——— & H. G. Richter. 1997. Systematic wood anatomy of *Myodocarpus*, *Delarbrea*, and *Pseudosciadium* (Araliaceae). Adansonia, sér. 3., 19: 61–75.

Philipson, W. R. 1970. A redefinition of *Gastonia* and related genera (Araliaceae). Blumea 18: 499–505.

———. 1978. A synopsis of the Malesian species of *Polyscias* (Araliaceae). Blumea 24: 169–172.

———. 1979. Araliaceae. Fl. Malesiana, ser. 1, 9: 1–105.

Plunkett, G. M. 1994. A Molecular-Phylogenetic Approach to the "Family-Pair Dilemma" in Apiales and Cyperales. Ph.D. Dissertation, Washington State University, Pullman, Washington.

———. 2001. Relationships of the order Apiales to subclass Asteridae: A re-evaluation of morphological characters based on insights from molecular data. Edinburgh J. Bot. 58 (in press).

——— & P. P. Lowry II. 2001. Relationships among "ancient araliads" and their significance for the systematics of Apiales. Molec. Syst. Evol. (in press).

———, D. E. Soltis & P. S. Soltis. 1996. Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbc*L sequences. Amer. J. Bot. 83: 499–515.

———, ——— & ———. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbc*L sequence data. Amer. J. Bot. 84: 565–580.

Raven, P. H. & D. I. Axelrod. 1972. Plate tectonics and southern hemisphere biogeography. Pp. 3–24 *in* K. Larsen & L. B. Holm-Nielsen (editors), Tropical Botany. Academic Press, London.

——— & ———. 1974. Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 61: 539–673.

Schlessman, M. A., D. G. Lloyd & P. P. Lowry II. 1990. Evolution of sexual systems in New Caledonian Araliaceae. Mem. New York Bot. Gard. 55: 105–117.

———, G. M. Plunkett, P. P. Lowry II & D. G. Lloyd. 2001. Sexual systems in New Caledonian Araliaceae: A preliminary phylogenetic reappraisal. Edinburgh J. Bot. 58 (in press).

Seemann, B. 1868. Revision of the natural order Hederaceae, being a reprint, with numerous additions and corrections, of a series of papers published in the Journal of Botany, British and Foreign. L. Reeve, London.

Smith, A. C. 1985. Flora Vitiensis Nova, Vol. 3. Pacific Tropical Botanical Garden, Lawai, Hawaii.

Soltis, D. E. & P. S. Soltis. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. Pp. 1–42 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II: DNA Sequencing. Klewer Academic Publishers, Boston.

———, ———, T. G. Collier & M. L. Edgerton. 1991. The *Heuchera* group (Saxifragaceae): Evidence for chloroplast transfer and paraphyly. Amer. J. Bot. 78: 1091–1112.

Soltis, P. S. & R. K. Kuzoff. 1993. ITS sequence variation within and among populations of *Lomatium grayi* and *L. laevigatum* (Umbelliferae). Molec. Phylogenet. Evol. 2: 166–170.

Stone, B. C. 1965a. Notes on *Polyscias* (Araliaceae) from Micronesia. Micronesica 2: 51–59.

———. 1965b. Notes on the type species of *Polyscias* J. R. and G. Forst. (Araliaceae). Taxon 14: 281–285.

Tajima, F. & M. Nei. 1984. Estimation of evolutionary distance between nucleotide sequences. Molec. Biol. Evol. 1: 269–285.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins. 1997. The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24: 4876–4882.

Tseng, C. & C. Hoo. 1982. A new classification scheme for the family Araliaceae. Acta Phytotax. Sinica 20: 125–129 [in Chinese].

Vargas, P., B. G. Baldwin & L. Constance. 1998. Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). Proc. Natl. Acad. Sci. U.S.A. 95: 235–240.

Viguier, R. 1906. Recherches anatomiques sur la classification des Araliacées. Ann. Sci. Nat. Bot. IX, 4: 1–210.

———. (1910–1913) 1925. Contribution à l'étude de la flore de la Nouvelle-Calédonie: Araliacées. J. Bot. (Morot) II, 3: 38–101. [Dated 1910–1913, but not distributed until 1925; cf. M. L. Green in Bull. Misc. Inform. 1928: 155–156. 1928.]

Webb, J. J. & J. G. Tracey. 1981. Australian rainforests: Patterns and change. Pp. 605–694 *in* A. Keast (editor), Ecological Biogeography of Australia, Vol. 1. Junk, The Hague, Boston, London.

Wen, J. & E. A. Zimmer. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. Molec. Phylogenet. Evol. 6: 167–177.

———, G. M. Plunkett, A. D. Mitchell & S. J. Wagstaff. 2001. The evolution of Araliaceae: A phylogenetic analysis based on ITS sequences of nuclear ribosomal DNA. Syst. Bot. 26: 144–167.

White, T. J., T. Bruns, S. Lee & J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in* M. A. Innis (editor), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.

Yokota, Y., T. Kawata, Y. Iida, A. Kato & A. Tanifuji. 1989. Nucleotide sequences of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. J. Molec. Evol. 29: 294–301.

EVOLUTION, PHYLOGENY, AND SYSTEMATICS OF THE JUGLANDACEAE[1]

Paul S. Manos[2] *and Donald E. Stone*[2]

ABSTRACT

A comprehensive systematic investigation was conducted on the extant Juglandaceae based on 25 species representing a broad sample of generic and infrageneric diversity. A total of 206 phylogenetically informative characters derived from morphological, chemical, chromosomal, and sequence-based studies formed the basis for comparative studies. Phylogenetic analysis was used to infer relationships and examine patterns of convergence in key biochemical and morphological traits associated with dispersal biology. Separate and combined parsimony analyses of three previously unpublished data sets (ITS, chloroplast DNA, morphology/chemistry) supported two major clades, Juglandoideae and Engelhardioideae, in agreement with a recent subfamilial classification. Within Engelhardioideae, the genus *Engelhardia* was found to be paraphyletic, as *E. roxburghiana* of the monotypic section *Psilocarpeae* was resolved as sister taxon to a New World subclade composed of *Oreomunnea* + *Alfaroa*. Within Juglandoideae, two tribes are recognized: Platycaryeae and Juglandeae. The monotypic genus *Platycarya* formed the sister group to Juglandeae, which was resolved fully (*Carya*–(*Juglans*–(*Cyclocarya* + *Pterocarya*))). Two new subtribes, Juglandinae and Caryinae, are described based on the cladistic pattern. Unique morphological apomorphies were detected for all genera, including the previously little-studied *Cyclocarya*, which was also determined to possess a novel base chromosome number for the family (N = 28). The nested position of *Annamocarya sinensis* within Old World *Carya*, combined with its lack of unique apomorphies suggested sectional recognition within *Carya* might be more appropriate for this taxon. Phylogenetic context was used to interpret patterns of morphological and chemical variation associated with the evolution of seed dispersal and the tropical versus temperate habitat. Although the syndrome of wind dispersal appears to be ancestral within the family, four novel origins of wing tissue are represented by *Engelhardia/Oreomunnea*, *Platycarya*, *Pterocarya*, and *Cyclocarya*. The convergence on animal dispersal has been achieved through three different developmental pathways in the production of a husk in *Alfaroa*, *Carya*, and *Juglans*. In general, wind-dispersed seeds have epigeal germination and those that are animal-dispersed are hypogeous, but *Oreomunnea* and *Cyclocarya* are exceptions in their respective clades by having wind-dispersed seeds with hypogeal germination. The seed-energy reserves are also revealing. With the exception of *Oreommunea*, wind-dispersed seeds have relatively high concentrations of the unsaturated linolenic (C) and linoleic (B) fatty acids (CB pattern), whereas all animal-dispersed fruits (viz., *Alfaroa*, *Carya*, and *Juglans*), and *Oreomunnea*, have relatively high concentrations of the unsaturated oleic (A) and linoleic (B) fatty acids (BA or AB pattern). Tropical genera, whether wind- or animal-dispersed (viz., *Oreomunnea*, *Alfaroa*, *Annamocarya*), have relatively high concentrations of the saturated palmitic fatty acid. Conversely, wind- and animal-dispersed fruits of temperate genera (viz., *Carya*, *Juglans*, *Cyclocarya*, *Pterocarya*, and *Platycarya*) have relatively low percentages of palmitic acid. The explanation here is based on the fact that seed fats must be fluid at the temperature of the living plant, thus selecting for saturated fats in warm tropical climates and unsaturated lipids in cool temperate climates.

Key words: *Cyclocarya*, ecological adaptations, Fagales, germination syndrome, Juglandaceae, phylogeny, taxonomy.

The Juglandaceae are generally considered to be a close-knit family of trees with pinnately compound leaves bearing glandular peltate scales, unisexual flowers, and wind-borne pollen. While no

[1] Support for this work was provided by NSF grants DEB 9707945 (PSM) and GB 5233X (DES). We thank a host of people who have contributed information to this project, which, in the case of DES, has stretched out over 40 years. Foremost on the list is Wayne E. Manning, who has been an inspiration, colleague, and friend over these many years; to him we dedicate this paper. Some of the collections for analysis have been graciously provided by L. J. Grauke of the USDA Pecan Research Station, Somerville, Texas; W. A. Haber of Monteverde, Costa Rica; K. Wurdack of the University of North Carolina, Chapel Hill; the Arnold Arboretum of Harvard University; and the J. C. Ralston Arboretum of North Carolina State University. Gas chromatographic analyses of the seed oils were performed by long-term colleague and dear friend George A. Adrouny, formerly of Tulane University, but now deceased, and M. E. Hohn, formerly of Indiana University. The SEM photomicrographs were made with the professional assistance of L. Eibest, Duke University. The drawing of *Annamocarya* in Figure 9 is the artistry of C. Zartman, Duke University. Translations of the Russian articles were contributed by B. M. Johnson, Duke University, while the Chinese translations were handled by Y. Tao, Duke University. Thanks are due to C. Geilselman, K. Hancock, M. E. Jones, and E. Kuhn for providing technical support in obtaining sequence data. Information on nutshell sclerenchyma was made available by S. R. Manchester, Florida Museum of Natural History, along with his technical expertise in review of the morphological matrix. Help in deciphering the suprageneric nomenclature received the serious attention of R. L. Wilbur, Duke University, and W. L. Culberson, Duke University, graciously provided the Latin descriptions. And finally, reviewers P. S. Herendeen, S. R. Manchester, and J. F. Smith deserve special thanks for their critical insight and extremely helpful comments.

[2] Department of Biology, Duke University, Durham, North Carolina 27708, U.S.A.

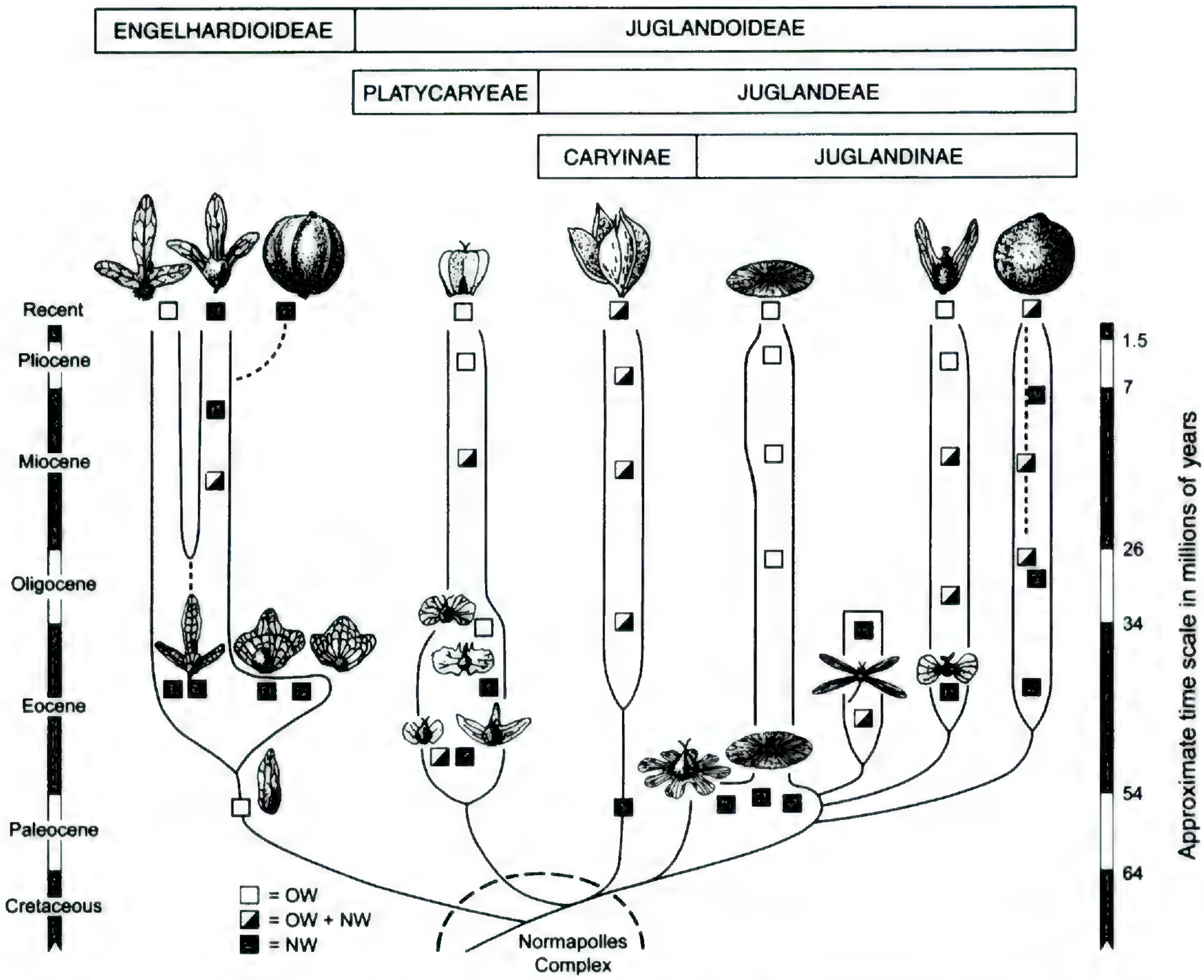


Figure 1. Evolutionary hypothesis of the phylogeny of extant and fossil Juglandaceae (adapted from Manchester, 1987, and pers. comm., and adjusted to show the higher-order taxonomy and relationships proposed in this paper). The fruits of extant taxa depicted across the top are, from left to right: *Engelhardia, Oreomunnea, Alfaroa, Platycarya, Carya, Cyclocarya, Pterocarya,* and *Juglans.* Extinct juglandaceous fruits based on their appearance in the fossil record are, from left to right and bottom to top: *Casholdia microptera, Palaeocarya puryearensis, Paleooreomunnea stoneana, Paraengelhardia eocenica* (Engelhardoideae); *Platycarya americana*; *Paleoplatycarya wingii*; *Hooleya lata*; *Hooleya hermis* (Juglandoideae: Platycaryeae); *Polyptera manningi, Cyclocarya brownii, Cruciptera simsonii,* and *Pterocarya macginitii* (Juglandoideae: Juglandeae: Juglandinae). OW = Old World; NW = New World.

new evidence has challenged this view, two things have become abundantly clear: The Juglandaceae are sister to the Rhoipteleaceae (Manos & Steele, 1997) and within the Juglandaceae there is a remarkably consistent evolutionary picture of the genera based on detailed phylogenetic studies incorporating data from morphology, anatomy, cytology, chemistry, and nucleotide sequences from both nuclear and chloroplast DNA (cpDNA). Phylogenetic analyses provide a much clearer picture of intrafamilial relationships than those presented by Manchester (1987) based on fossil history and by Manning (1978), Schaarschmidt (1985), and Iljinskaya (1990) using morphology, and validate Smith and Doyle's (1995) recognition of two major clades within the family (viz., Engelhardioideae and Juglandoideae; see Fig. 1 and Results).

The family consists of seven to ten extant genera (Table 1) and 60+ extant species located mainly in the Northern Hemisphere of both the Old and New World, though their present-day distribution belies the true range based on historical information (Manchester, 1987, 1999; see Fig. 1). *Annamocarya* (= *Carya sinensis* Dode), *Cyclocarya* [= *Pterocarya paliurus* (Batalin) Iljinskaya], *Engelhardia* [including *E. roxburghiana* Wallich = *Alfaropsis roxburghiana* (Wallich) Iljinskaya], *Platycarya,* and *Pterocarya* are strictly Old World, *Alfaroa* and *Oreomunnea* are New World, whereas *Carya* and *Juglans* occur in both regions (Lu, 1982). *Engelhardia* in the Old World and *Juglans* in the New World both extend into the Southern Hemisphere, with the former ranging into Sumatra, Java, and New Guinea and the latter extending from southern Canada to

Table 1. Contemporary classifications of the Juglandaceae recognizing subfamilies, tribes, subtribes, and genera.

Leroy (1955)	Manning (1978)	Schaarschmidt (1985)	Manchester (1987)	Iljinskaya (1990, 1993)	Manos and Stone (this article)
I. OREOMUNNOIDEAE	I. PLATYCARYOIDEAE	I. PLATYCARYOIDEAE	I. PLATYCARYOIDEAE	I. PLATYCARYOIDEAE	I. ENGELHARDIOIDEAE
1. *Platycarya*	1. *Platycarya*	PLATYCARYEAE	PLATYCARYEAE	1. *Platycarya*	4. *Engelhardia*
3. *Pterocarya*		1. *Platycarya*	1. *Platycarya*		5. *Oreomunnea*
4. *Engelhardtia*	II. JUGLANDOIDEAE	7. *Carya*		II. ENGELHARDIOIDEAE	6. *Alfaroa*
5. *Oreomunnea*	JUGLANDEAE		ENGELHARDIEAE	4. *Engelhardia*	[9. *Alfaropsis*]
6. *Alfaroa*	2. *Juglans*	ENGELHARDIEAE	4. *Engelhardia*	5. *Oreomunnea*	
	3. *Pterocarya*	4. *Engelhardia*	5. *Oreomunnea*	6. *Alfaroa*	II. JUGLANDOIDEAE
II. JUGLANDOIDEAE		5. *Oreomunnea*	6. *Alfaroa*	9. *Alfaropsis*	PLATYCARYEAE
2. *Juglans*	ENGELHARDIEAE	6. *Alfaroa*			1. *Platycarya*
7. *Carya*	4. *Engelhardia*		II. JUGLANDOIDEAE	III. JUGLANDOIDEAE	
10. *Annamocarya*	5. *Oreomunnea*	II. JUGLANDOIDEAE	JUGLANDEAE	JUGLANDEAE	JUGLANDEAE
	6. *Alfaroa*	2. *Juglans*	2. *Juglans*	2. *Juglans*	JUGLANDINAE, subtr. nov.
		3. *Pterocarya*	3. *Pterocarya*	3. *Pterocarya*	2. *Juglans*
	HICORIEAE	8. *Cyclocarya*	8. *Cyclocarya*	8. *Cyclocarya*	3. *Pterocarya*
	7. *Carya*				8. *Cyclocarya*
	(incl. *Annamocarya*)		HICOREAE	HICORIEAE	
			7. *Carya*	7. *Carya*	CARYINAE, subtr. nov.
					7. *Carya*
					[10. *Annamocarya*]

northern Argentina. When the very extensive fossil record is included, however, the majority of these genera have Tertiary distributions recorded in Asia, Europe, and North America, with only *Alfaroa* and *Annamocarya* lacking a fossil record (Manchester, 1987). North America is the epicenter of fossil diversity of the family, and Asia appears to be a refugium for many of the modern members (Stone, 1989; Manchester, 1999).

In light of the conservative nature of the vegetative features, taxonomy of the genera is based largely on flower and fruit characters, though pollen, pith, and tracheary elements play a role in recognizing related groups of genera (Manning, 1978; Manchester, 1987). The basic ground plan of the pistillate flowers consists of a bract, two bracteoles, four sepals, and a bicarpellate inferior ovary with a bifurcate style/stigma (Fig. 4). Variations on a theme involving differential growth, orientation, connation, adnation, and deletion of floral parts are reflected in the mature fruits (Fig. 1). They vary from the tiny (6 mg) wind-borne seeds of *Platycarya*, to the large (14+ g) animal-dispersed fruits of *Alfaroa*, *Carya*, and *Juglans*, from 2-winged (*Platycarya*, *Pterocarya*) to 3-winged (*Engelhardia*, *Oreomunnea*) to circular-winged (*Cyclocarya*), and from nuts where the husk is derived from the bract, bracteoles, and sepals (*Juglans*), to those derived from the bract/bracteoles (*Annamocarya*, *Carya*), to those where only the sepals play a role (*Alfaroa*) (Manning, 1978; Stone, 1989). Although not tested by cladistic analysis, the evidence from comparative morphology led to the suggestion that winged fruits had four separate origins and that the animal-dispersed nut has evolved independently three times (Stone, 1973). In spite of these apparent convergences, the floral diagrams in combination with a few other diagnostic characters have provided the basis for recognition of two or three subfamilies (though not necessarily with the same genera) and two to four tribes (Table 1).

Traditional and Current Concepts of Inter- and Intrafamilial Relationships

The Juglandaceae were traditionally part of the amentiferous concept (Stern, 1973) characterized by apetalous, unisexual flowers and wind pollination, and included such orders as the Casuarinales, Fagales, Garryales, Juglandales, Leitneriales, Myriciales, Salicales, and Urticales. While the artificiality of this group has long been recognized, the debate shifted some 30 years ago to the Hamamelididae with the circumscription of the subclass by Cronquist (1968, 1981) and Takhtajan (1968, 1980) that included the Trochodendrales, Hamamelidales, Eucommiales, Urticales, Leitneriales, Juglandales, Myricales, Fagales, and Casuarinales. To include the Juglandales in the Hamamelididae requires a leap of faith, however, because the leaves of both the Juglandaceae and Rhoipteleaceae are pinnately compound, unique features in this subclass. For this reason Wolfe (1973) associated these two families more closely with the Rosidae. And Thorne (1973), as well, saw relationships of the Juglandales elsewhere, citing the Anacardiaceae and Burseraceae as fellow members of the superorder Rutiflorae, order Rutales.

Resolution of the best placement of the Juglandales started with both cladistic analysis of the "Lower" Hamamelididae (i.e., Trochodendrales and Hamamelidales: Hufford & Crane, 1989) and consideration of the origins of the Fagaceae (Nixon, 1989). In the latter study, the Juglandaceae, Rhoipteleaceae, Myricaceae, Nothofagaceae, Betulaceae, and Casuarinaceae were recognized as part of the "Higher" Hamamelididae based in part on the possession of porate pollen with granular walls, a derived state of the tricolporate grains found in Fagaceae and more typical Rosidae (Walker & Doyle, 1975; Zavada & Dilcher, 1986). This distinction within Hamamelididae was later followed up by Hufford (1992: 221) in a cladistic study of the Rosidae in which he concluded that the "Lower" Hamamelididae "are a paraphyletic assemblage at the base of the Rosidae, and the "Higher" Hamamelididae (i.e., Fagales, Juglandales, and Casuarinaceae) are nested among Rosidae." This placement was subsequently supported by comparative molecular studies of cpDNA from *Juglans* (Manos et al., 1993) and *rbc*L data from *Carya* (Chase et al., 1993; Qiu et al., 1993, 1998). With the developing notion of a Rosid placement for Juglandaceae, Gunter et al. (1994) examined the *rbc*L gene in *Carya*, *Juglans*, and *Pterocarya*, in combination with chemical and morphological information, and concluded that there is a closer relationship to the Betulaceae, Casuarinaceae, Fagaceae, and Myricaceae, than to Anacardiaceae (sensu Thorne, 1983). The most recent and comprehensive evaluation of the relationships of the Juglandaceae has come from the phylogenetic analysis of the "Higher" Hamamelididae by Manos and Steele (1997) using the cpDNA gene, *mat*K. In this paper the authors examined *Alfaroa*, *Carya*, and *Juglans* and provided the first sequence-based analysis for *Rhoiptelea* (also see Chen et al., 1998). These three members of the Juglandaceae were resolved as a sister group to *Rhoiptelea*, and they in turn formed a sister clade

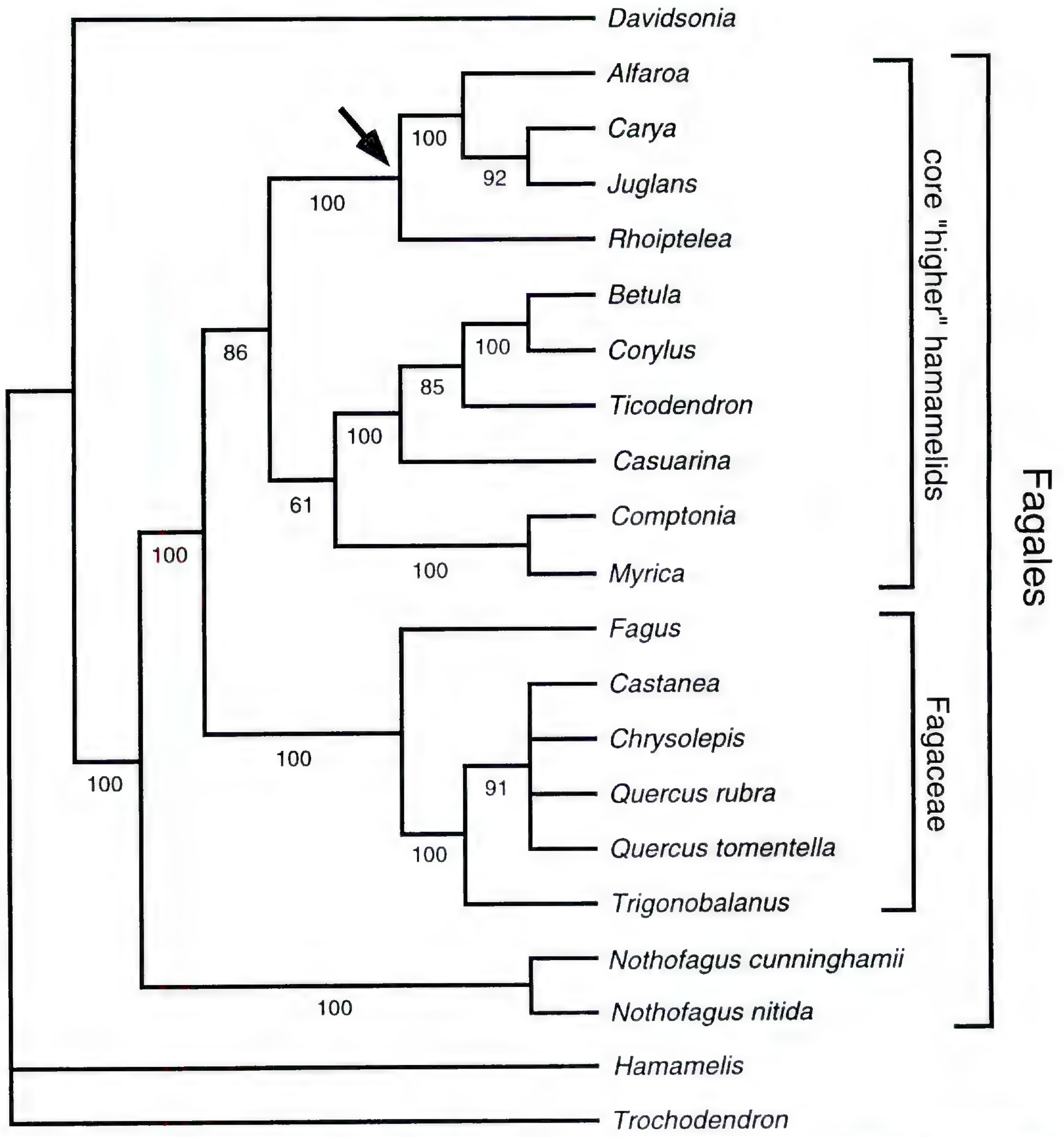


Figure 2. Cladogram of the "higher" Hamamelididae based on plastid sequence data (from Manos & Steele, 1997).

with the Betulaceae, Ticodendraceae, Casuarinaceae, and Myricaceae (Fig. 2).

The intrafamilial classification of the Juglandaceae has had an interesting history as well (Manning, 1978). For the purposes of this paper Manning's framework (Table 1), based largely on seminal morphological studies on the inflorescence (1938), pistillate flowers (1940), and staminate flowers (1948), will be used as a baseline for evaluating other proposals. The principal subdivisions (subfamilies) of the Juglandaceae, according to Manning, are the Platycaryoideae, with the monotypic *Platycarya*, and the Juglandoideae, which includes everything else. As much as anything, this classification was an attempt to deal with the autapomorphic characteristics of *Platycarya* such as the cone-like infructescence, pollen with pseudocolpi, and vascular tracheids. In a published abstract Bolick (1983) reported that her cladistic analysis based on morphological characters was congruent with Manning's subfamily classification. Schaarschmidt (1985), on the other hand, using the full range of morphological characters from extant and fossil material, concluded that *Platycarya* is closely related to *Carya*, and that Platycaryoideae contained two sister groups, the Platycaryeae with *Platycarya* and *Carya* and Engelhardieae with *Engelhardia*, *Oreomunnea*, and *Alfaroa*. The Juglan-

doideae in his scheme are comprised of *Juglans*, *Pterocarya*, and *Cyclocarya* (Table 1). In 1990 Iljinskaya used the same basic information to recognize three subfamilies: Platycaryoideae, Engelhardoideae, and the Juglandoideae.

The time was clearly ripe in 1995 when Smith and Doyle conducted the first comprehensive cladistic analysis of the family based on molecular and morphological data. Although the sampling of Juglandaceae was limited to seven of the 60+ taxa, they had representation from the majority of the genera, including *Alfaroa*, *Carya*, *Engelhardia*, *Juglans*, *Oreomunnea*, *Platycarya*, and *Pterocarya*. What they found as several others had before is that the Juglandaceae comprise two major clades. The notable difference they proposed, however, is that *Engelhardia*, *Oreomunnea*, and *Alfaroa* are united in one clade and *Platycarya* is positioned as a sister group to *Juglans*, *Pterocarya*, and *Carya* in the second clade. Of the many intrafamilial relationships that have been proposed over the years, the findings by Smith and Doyle and the classification by Iljinskaya are the only ones giving subfamilial recognition to *Engelhardia*, *Oreomunnea*, and *Alfaroa*. Our challenge has been to assess these various proposals by examining a far larger and more diverse data set.

This study of 25 juglandaceous taxa across 10 genera (including *Alfaropsis*, *Annamocarya*, and *Cyclocarya*) provides new information on morphology, anatomy, cytology, chemistry, and DNA sequences and combines these data with previously published results for an in-depth cladistic analysis. The opportunity presents itself therefore to evaluate the relative contribution of the molecular and nonmolecular characters, to examine the validity of more problematic genera such *Alfaropsis*, *Annamocarya*, and *Cyclocarya*, and to explore morphological and chemical evolution, and in particular, apparent convergence in seed fat composition, fruit dispersal, and seedling germination.

Materials and Methods

TAXA

Plants were collected from a combination of sources, notably natural populations and accessions of the USDA Pecan Research Station, Somerville, Texas, the Arnold Arboretum, Boston, Massachusetts, and the J. C. Ralston Arboretum, Raleigh, North Carolina (Table 2). Total genomic DNA was isolated from fresh and silica gel-dried plant material using the DNeasy Plant Mini Kit (Qiagen; Valencia, California). Sampling included 25 of the approximately 60 species recognized within the family and all infrageneric sections of 10 genera. On the basis of various lines of supporting evidence ranging from classical morphology to molecular phylogenetics, *Rhoiptelea chiliantha* (Rhoipteleaceae) was employed as the outgroup (Manning, 1938, 1940; Withner, 1941; Stone & Broome, 1971; Oginuma et al., 1995; Manos & Steele, 1997; Chen et al., 1998).

CHARACTERS

Morphological character state variation was studied intensively and extensively using a combination of fresh, liquid-preserved, and dried herbarium material. Several characters are recorded for the first time, and character state variation and coding issues are discussed (see Results). The morphology of the lower leaflet surface was determined from herbarium samples soaked in Hoyer's solution for 24 hours (see Hardin, 1981), dehydrated in an alcohol series, critical point dried, coated, and examined with a Philips 501 SEM. The fatty acid content of the fruits was assayed following the methods of Stone et al. (1965, 1969). The chromosome counts were made on pollen mother cells fixed in three parts ethanol to one part glacial acetic acid, squashed and stained in acetocarmine, and then photographed with phase contrast microscopy.

Molecular techniques for the direct sequencing of the nuclear ribosomal ITS region followed Manos et al. (1999), whereas chloroplast DNA intergenic spacer sequences (*rbc*L/*atpb*E and *trn*L/*trn*F) were obtained using standard techniques and the primers designed by Hodges and Arnold (1994) and Taberlet et al. (1991). Alignments were performed manually. The three data sets generated in this study (morphology-chemical, cpDNA, and ITS) are available by request from the authors and are deposited in TREEBASE (http://www.herbaria.harvard.edu/treebase/index.html).

DATA ANALYSIS

Equally weighted parsimony analyses were conducted on the separate and combined data sets with PAUP* version 4.0b3a (Swofford, 2000) using BRANCH AND BOUND and 1000 random entry sequences. Trees were evaluated for standard measures of fit and resolving power, as measured by the total number of nodes supported in the strict consensus. Incongruence between data sets was quantified using the Mickevich and Farris incongruence index (I_{MF}; Mickevich & Farris, 1981), which measures the relative degree of disparity between data sets using tree length difference. Incongruence also was evaluated using the incongruence length difference test of Farris et al. (ILD; 1994), as imple-

mented in PAUP*. The hypothesis of congruence was tested by calculating the length difference among resampled partitions from the pool of characters in the combined original matrices. Incongruence between data sets is identified if the additive tree lengths taken from resampled matrices are greater than the sum of the tree lengths from the original data. Bootstrap analysis (Felsenstein, 1985) using 1000 replicates was used to measure relative support. The distribution of morphological apomorphies was explored under *acctran* and *deltran* optimization using the reconstructions provided by PAUP*. Internal nodes were evaluated for the relative contribution of morphological and/or molecular support using the proportion of support contributed from each data set in combined analysis as a percentage value of the total branch length.

Results

Morphological-chemical character analysis

This study revealed a significant number of new characters, as well as an improved interpretation of character state variation based on more complete sets of observations. A matrix composed of 50 characters was assembled (Table 3), and 38 of these were the basis of subsequent phylogenetic analysis. Missing data are indicated by "?"; the character state followed by (?) signifies a highly probable but unknown condition; and NA in the case of the outgroup *Rhoiptelea* notes that these character-state variations relating to the inflorescence and unisexual flowers of the Juglandaceae are not applicable. In the analysis below, the discussion of the principal characters encompasses most of the variation found at both the genus and species level. Exceptions to the general generic mode of variation have resulted in the recognition of a plethora of taxonomic subgenera and sections. The subfamilial terminology summarized in Table 2 generally follows that of Manning (1978).

I. Vegetative characters

1. Tree phenology: strictly deciduous (0); evergreen (1). The temperate genera are deciduous, and tropical members are generally evergreen, but interspecific variability occurs. In *Engelhardia spicata*, for example, from the montane Himalayas, southern China, Cambodia, Malaysia, and Borneo, the tree is "often deciduous for a short time and then flowering, not in definite periods" (Jacobs, 1960: 153). On the other hand, *Annamocarya sinensis* (= *Carya sinensis* Dode) from southern China and northern Vietnam, is probably more representative of the tropical evergreen condition. While the tree has persistent leaves (Chevalier, 1941), new growth flushes at the same time that old leaves are still present (Grauke et al., 1991). This timing is true as well for the New World *Alfaroa* and *Oreomunnea* (Stone, pers. obs.).

2. Terminal buds: strictly naked (0); with bud scales (1). Genera characterized by bud scales may also have some taxa with naked buds. Both types, for example, are found in *Pterocarya*: section *Pterocarya* has naked terminal buds (e.g., *P. stenoptera*), whereas section *Platyptera* has 2 to 4 caducous scales (e.g., *P. macroptera* and *P. rhoifolia*). Similarly, *Carya* sect. *Apocarya* has pseudo-valvate scales, those of section *Carya* are imbricate, and section *Sinocarya* has naked terminal buds (Lu et al., 1999).

3. Branchlet pith: solid (0); chambered (1). Chambered pith characterizes *Juglans*, *Pterocarya*, and *Cyclocarya* of the tribe Juglandeae (Manning, 1978; see also Manchester, 1987). Solid pith is the rule in the other members of Juglandaceae, as well as the Rhoipteleaceae (Tang, 1932).

4. Stipules: present (0); absent (1). A pair of thick, papery, caducous stipules flank a swollen petiole base in *Rhoiptelea* (Wu & Kubitzki, 1993), but are absent in the Juglandaceae.

5. Leaf phyllotaxy: alternate (0); opposite (1). Alternate phyllotaxy generally characterizes the family. However, the compound leaves of *Alfaroa* and *Oreomunnea* are principally opposite (decussate), though whorled and alternate arrangements occur occasionally, but mainly on sucker shoots (Stone, 1977).

6. Leaflet arrangement: odd-pinnate (0); even-pinnate (1). Odd-pinnate (imparipinnate) compound leaves are characteristic of the Juglandoideae containing mainly taxa of the temperate zone. Even-pinnate is a feature shared by the extant representatives of the Engelhardioideae. Note, however, that "the few Eocene complete leaves seen so far are odd-pinnate" (Manchester, pers. comm.; Dilcher & Manchester, 1986; Manchester, 1987).

7. Leaflet margin: serrate (0); strictly entire (1). Genera in which the majority of species have serrate leaflets may also have a few with entire leaflets. In the case of *Engelhardia* sect. *Engelhardia*, *E. rigida* has entire leaflets whereas those of *E. serrata* are more or less distinctly serrate or crenate (Jacobs, 1960). Similarly in *Juglans*, *J. regia* of section *Juglans* has entire leaflets, whereas species in the other three sections have serrate ones. Entire margins are characteristic of the leaflets from adult trees of all tropical members, including *Engelhar-*

Table 2. Voucher information for samples of Rhoipteleaceae and Juglandaceae sequenced in this study. GenBank accession numbers are provided in the following order—chloroplast DNA spacer regions: *rbc*L/*atb*E, *trn*L, and nuclear rDNA internal transcribed spacers (ITS).

Taxa	Vouchers	GenBank accession nos.
Rhoiptelea *chiliantha* Diels & Handel-Mazzetti	CHINA. Yunnan: *Kelly 1017* (BH)	AF303746, AF303773, AF303800
Alfaroa *costaricensis* Standley	COSTA RICA. San José: *Stone 4087* (DUKE)	AF303749, AF303776, AF303803
A. guanacastensis Stone	COSTA RICA. Alajuela: *Stone 4085* (DUKE)	AF303750, AF303777, AF303804
A. manningii Léon	COSTA RICA. Cartago: *Stone 4083, 4084* (DUKE)	AF303751, AF303778, AF303805
A. williamsii A. Molina	COSTA RICA. Cartago: *Stone 3971* (DUKE)	AF303752, AF303779, AF303806
Annamocarya *sinensis* (Dode) Leroy	VIET NAM. Nho Quan: *Stone 4066* (DUKE). Seed grown at USDA Pecan Research Station (*Grauke et al. 90-SIN-VN-2*), Somerville, TX	AF303764, AF303791, AF303818
Carya* sect. *Sinocarya		
C. cathayensis Sargent	CHINA. Anhwei: *Stone 4093* (DUKE). Seedling graft on pecan rootstock grown at USDA Pecan Research Station (*CSL-Bay 9*), Somerville, TX	AF303765, AF303792, AF303819
C. tonkinensis Lecomte	VIET NAM. Son La: *Stone 4098* (DUKE). Seed grown at USDA Pecan Research Station (*Grauke et al. GO-TON-VN 4.1*), Somerville, TX	AF303772, AF303799, AF303826
C.* sect. *Apocarya		
C. cordiformis (Wangenheim) K. Koch	U.S.A. North Carolina, Pender: *Stone 4056* (DUKE)	AF303766, AF303793, AF303820
C. illinoinensis (Wangenheim) K. Koch	U.S.A. North Carolina, Durham: *Stone 4048* (DUKE)	AF303771, AF303798, AF303825
C.* sect. *Carya		
C. myristiciformis (F. Michaux) Nuttall	U.S.A. North Carolina, Pender: *Stone 4050* (DUKE)	AF303767, AF303794, AF303821
C. ovata (Miller) K. Koch	U.S.A. North Carolina, Pender: *Stone 4053* (DUKE)	AF303768, AF303795, AF303822
C. glabra (Miller) Sweet	U.S.A. North Carolina, Durham: *Stone 4097* (DUKE)	AF303769, AF303796, AF303823
C. tomentosa (Poiret) Nuttall	U.S.A. North Carolina, Durham: *Stone 4096* (DUKE)	AF303770, AF303797, AF303824
Cyclocarya *paliurus* (Batalin) Iljinskaya	U.S.A. North Carolina, Wake: *Wurdack E43 AA MA '91 = Stone 4075* (DUKE)	AF303763, AF303790, AF303817
Engelhardia* sect. *Psilocarpeae		
E. roxburghiana Wallich	TAIWAN. Tung: *Stone GH 111* (DUKE). Seed grown at Duke Univ., Durham, NC	AF303747, AF303774, AF303801
E.* sect. *Engelhardia		
E. spicata Leschenault ex Blume	CHINA. Yunnan: *Manos 1383* (DUKE).	AF303748, AF303775, AF303802
Juglans* sect. *Trachycaryon		
J. cinerea L.	U.S.A. New York, Westchester: *Manos 1341* (DUKE)	AF303759, AF303786, AF303813

Table 2. Continued.

Taxa	Vouchers	GenBank accession nos.
J.* sect. *Cardiocaryon		
J. mandshurica Maximowicz	CHINA. *Stone 4062* (DUKE). Seed secured from UC-Davis grown at USDA Pecan Research Station (CSHQ 7.5–14), Somerville, TX	AF303758, AF303785, AF303812
J.* sect. *Rhysocaryon		
J. microcarpa Berlandier	U.S.A. Texas, Uvalde: *Stone 4001* (DUKE). Seed grown at USDA Pecan Research Station (CSHQ 14–8), Somerville, TX	AF303757, AF303784, AF303811
J. nigra L.	U.S.A. New York, Tompkins: *Smith 263* (BH). Seed grown at Cornell University, Ithaca, NY	AF303756, AF303783, AF303810
J. sect. Juglans		
J. regia L.	CHINA: *Stone 4092* (DUKE). Graft secured from UC-Davis grown at USDA Pecan Research Station (CSHQ 14–12), Somerville, TX	AF303755, AF303782, AF303809
Oreomunnea *mexicana* (Stand.) Leroy	COSTA RICA. Cartago: *Stone 3974* (DUKE)	AF303753, AF303780, AF303807
Platycarya *strobilacea* Siebold & Zuccarini	U.S.A. North Carolina, Durham: *Stone 4071* (DUKE)	AF303754, AF303781, AF303808
Pterocarya sect. *Platyptera*		
P. macroptera var. *insignis* (Rehder & E. H. Wilson) Manning	CHINA. Qinling: *Stone 4064* (DUKE). Seed grown at USDA Pecan Research Station, Somerville, TX	AF303760, AF303787, AF303814
P.* sect. *Pterocarya		
P. stenoptera C. DC.	CHINA: *Stone 4063* (DUKE). Seed from UC-Davis grown at USDA Pecan Research Station (CSHQ 14-2), Somerville, TX	AF303761, AF303788, AF303815
	CHINA. Shennongjia Forest District: *Stone 4069* (DUKE). Seed grown at Arnold Arboretum (#1652-80-B), Jamaica Plain, MA	AF303762, AF303789, AF303816

dia sect. *Psilocarpeae*, *Oreomunnea* and *Alfaroa* (see Dilcher & Manchester, 1986), and *Annamocarya*. Some species of both *Oreomunnea* and *Alfaroa*, however, display serrate leaflets in the seedling and sapling stage as well as on sucker shoots of adult trees (Stone, 1977).

8. Texture of lower leaflet surface: smooth to verrucose (0); papillose (1). Examples of the former are found in the temperate members such as *Carya* (Hardin & Stone, 1984), *Juglans*, *Pterocarya*, and *Engelhardia* (Fig. 3A) of the Old World tropics. The New World tropical genera of *Alfaroa* and *Oreomunnea*, however, are papillose (see Fig. 3B; Dilcher, 1974; Dilcher & Manchester, 1986).

9. Druse crystals in leaf palisade: present (0); absent (1). Metcalfe and Chalk (1950) reported the occurrence of large crystal clusters in the leaf palisade. Specifically, druse crystals are found principally in temperate genera, although the tropical *Annamocarya* is similar in this respect to *Carya*;

Table 3. Cladistic matrix of morphological, chemical, and chromosomal characters for the Juglandaceae and Rhoipteleaceae taxa with unique character state combinations. NA = not applicable (see text and Fig. 9 for details); ? = unknown; (?) = unknown, but believed to exhibit state indicated.

Characters	*Engelhardia spicata*	*E. roxburghiana*	*Oreomunnea mexicana*	*Alfaroa* spp.	*Pterocarya stenoptera*	*P. macroptera*	*Cyclocarya paliurus*	*Juglans cinerea*	*J. regia*	*J. nigra*	*Annamocarya sinensis*	*Carya* spp.	*Platycarya strobilacea*	*Rhoiptelea*
1. Phenology: deciduous (0); evergreen (1)	1	1	1	1	0	0	0	0	0	0	1	0	0	0
2. Terminal buds: naked (0); with scales (1)	0	0	0	0	0	1	0	1	1	1	1	1	1	0
3. Pith: solid (0); chambered (1)	0	0	0	0	1	1	1	1	1	1	0	0	0	0
4. Stipules: present (0); absent (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	0
5. Phyllotaxy: alternate (0); opposite (1)	0	0	1	1	0	0	0	0	0	0	0	0	0	0
6. Leaflet arrangement: odd-pinnate (0); even-pinnate (1)	1	1	1	1	0	0	0	0	0	0	0	0	0	0
7. Leaflet margins: serrate (0); entire (1)	0	1	1	1	0	0	0	0	1	0	1	0	0	0
8. Leaflet surface: smooth (0); papillose (1)	0	0	1	1	0	0	0	0	0	0	0	0	0	0
9. Druse crystals: present (0); absent (1)	1	1	1	1	0	0	0	0	0	0	0	0	0	0
10. Rhomboid crystals: absent (0); present (1)	0	0	1	1	0	0	0	0	0	0	0	0	0	0
11. Wood porosity: ring/semi-ring (0); diffuse (1)	1	1	1	1	1	1	0	0	0	0	1	0	0	0
12. Vessel plates: scalariform (0); scalariform in part (1); simple (2)	1	1	1	1	2	2	2	2	2	2	2	2	2	0
13. Vessel element length: −650 μm (0); <650 μm (1)	0	0	0	0	1	1	1	1	1	1	1	1	1	0
14. Vessel pores: angular to round (0); round (1)	0	0	0	0	0	0	0	0	0	0	1	1	1	0
15. Inflorescences: androgynous (0); unisexual (1)	0	0	0	0	1	1	1	1	1	1	1	1	0	NA
16. Staminate catkins: clusters to single (0); single (1)	0	0	0	0	1	1	0	1	1	1	0	0	0	NA
17. Pistillate catkins: pendent (0); erect (1)	0	0	1	1	0	0	0	1	1	1	1	1	1	NA

Table 3. Continued.

Characters	*Engelhardia spicata*	*E. roxburghiana*	*Oreomunnea mexicana*	*Alfaroa spp.*	*Pterocarya stenoptera*	*P. macroptera*	*Cyclocarya paliurus*	*Juglans cinerea*	*J. regia*	*J. nigra*	*Annamocarya sinensis*	*Carya spp.*	*Platycarya strobilacea*	*Rhoiptelea*
18. Pistillate spikes: lax (0); condensed (1)	0	0	0	0	0	0	0	0	0	0	0	0	1	NA
19. Flowers: bisexual (0); unisexual (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	0
20. Staminate bracts: 3-lobed (0); 1-lobed (1)	0	0	0	0	1	1	1	1	1	1	1	1	1	NA
21. Staminate bract vasculature: 3 or more strands (0); single (1)	0	0	0	0	1	1	1	1	1	1	0	1	0	NA
22. Male flower bracteoles: present (0); absent (1)	0	0	0	0	0	0	0	0	0	0	0	0	1	NA
23. Male flower sepals: 4 (0); 2 (1); absent (2)	0	1	0	0	0	0	0	0	0	0	2	2	2	NA
24. Pollen pores: equatorial (0); subequatorial (1); heteropolar (2)	0	0	0	0	0	0	0	2	2	2	1	1	0	0
25. Pollen apertures: triporate (0); 4 pores or more (1)	0	0	0	0	1	1	1	1	1	1	0	0	0	0
26. Pollen apertures: elongate (0); oblong to circular (1)	1	0	1	1	1	1	1	1	1	1	1	1	0	0
27. Pollen sculpturing: pseudocolpi absent (0); present (1)	0	0	0	0	0	0	0	0	0	0	0	0	1	0
28. Pollen wall: exine not thinning (0); exine thinning (1)	0	0	0	0	0	0	0	1	1	1	1	1	0	0
29. Pollen size: 15 μm (0); 17–26 μm (1); >26 μm (2)	1	0	1	1	2	2	2	2	2	2	2	2	0	1
30. Pollen wall structure: nexine thick (0); nexine thin (1)	1	0	0	0	0	0	0	0	0	0	0	0	0	0
31. Ovary position: superior (0); inferior (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	0
32. Ovary: 2-loculed (0); 1-loculed (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	0
33. Carpel fusion: median (0); transverse (1)	0	0	0	0	0	0	0	0	0	0	1	1	1	0
34. Stigmatic disk: absent (0); present (1)	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Table 3. Continued.

Characters	*Engelhardia spicata*	*E. roxburghiana*	*Oreomunnea mexicana*	*Alfaroa spp.*	*Pterocarya stenoptera*	*P. macroptera*	*Cyclocarya paliurus*	*Juglans cinerea*	*J. regia*	*J. nigra*	*Annamocarya sinensis*	*Carya spp.*	*Platycarya strobilacea*	*Rhoiptelea*
35. Stigmas: carinal (0); split-carinal (1); commissural (2)	2	1	0	0	0	0	2	0	0	0	2	2	0	0
36. Stigma type: see text	3	2	1	1	0	0	0	0	0	0	4	4	0	4
37. Ovule type: anatropous (0); orthotropous (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	0
38. Ovules: bitegmic (0); unitegmic (1)	1	1	1	1	1	1	1	1	1	1	1	1	1	0
39. Pistillate bracts: free (0); attached (1)	1	1	1	1	1	1	1	1	1	1	1	1	0	NA
40. Pistillate bracts: large (0); small or fused to other parts (1)	0	0	0	1	1	1	1	1	1	1	1	1	0	NA
41. Pistillate bracts: trilobed (0); unlobed (1)	0	0	0	0	1	1	1	1	1	1	1	1	1	NA
42. Pistillate bracteoles/ovary: see text (1–5)	1	0	2	0	3	3	3	4	4	4	5	5	3	NA
43. Fruit types: simple (0); accessory, see text (1–7)	1	1	1	5	3	3	2	6	6	6	7	7	4	0
44. Fruit chambers at base: 2 (0); 2 or 4 (1); 8 (2)	1	1	2	2	1	1	1	0	1	1	0	1	0	0
45. Nutshell sclerenchyma: fibers (0); sclereids (1)	0	0	0	0	1	1	1	1	1	1	1	1	1	0
46. Cotyledons: epigeous (0); hypogeous (1)	0	0	1	1	0	0	1	1	1	1	1	1	0	1
47. Cotyledonary node: 1-gap (0); 2+ gaps (1)	0	0	0	0	0	0(?)	?	1	1	1	1(?)	1	0	?
48. Cotyledonary node: 2 traces (0); 3+ traces (1)	0	0	1	1	0	0	?	0	0	0	1(?)	1	0	?
49. Seed oils: see text (1–4)	1	1	2	2	0	0	0	3	3	3	2	4	0	0
50. Base chromosome number: $x = 16$ (0); $x = 15$ (1); $x = 28$ (2)	0	0	0	0	0	0	2	0	0	0	?	0	1	0

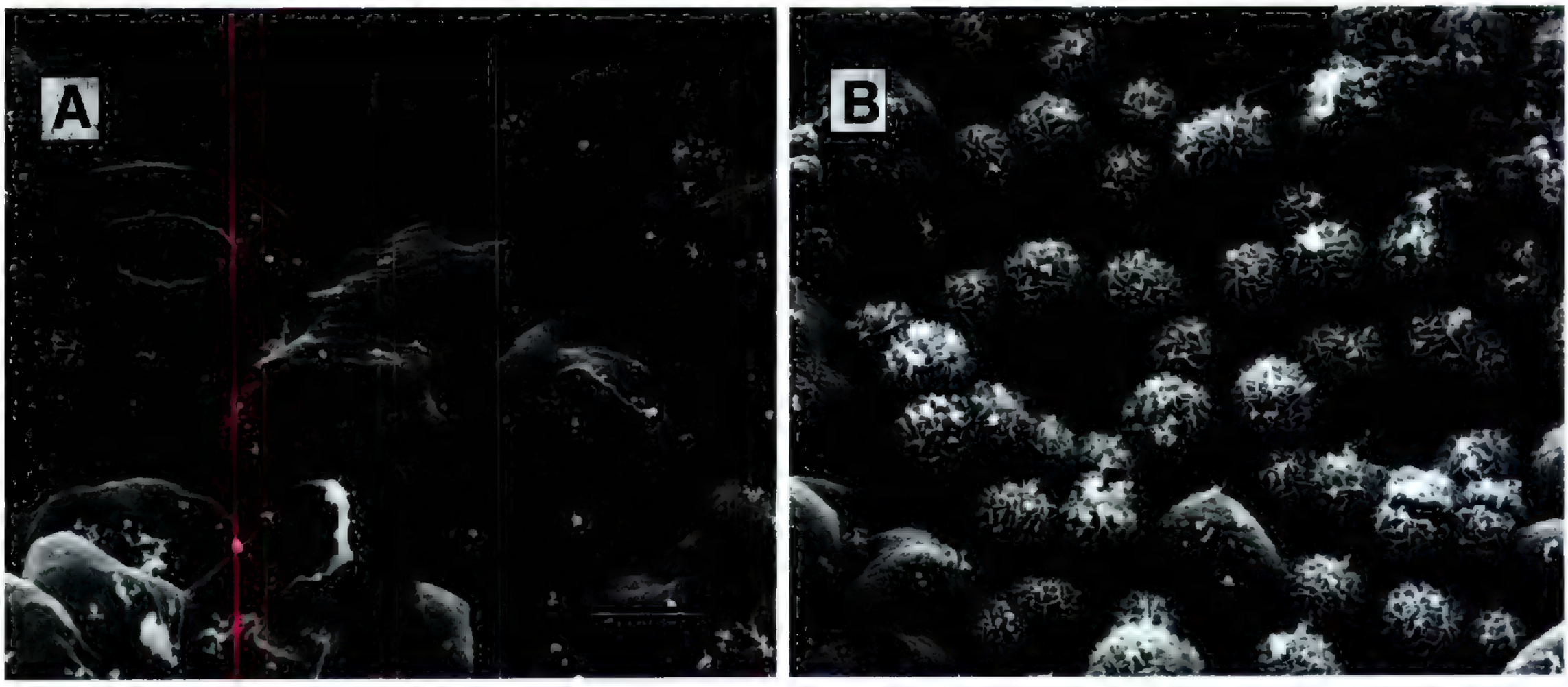


Figure 3. SEM photomicrographs of the lower leaflet surface of Old World *Engelhardia* and New World *Alfaroa*. —A. *Engelhardia spicata* var. *colebrookeana* (Lindl. ex Wall.) Koord. & Valet. (*Tsang 24*, UC 1346717) with smooth, undulating epidermal cells and slightly raised stomata. —B. *Alfaroa hondurensis* L. O. Williams ex Manning (*Stone 2185*, DUKE) with ca. 8 waxy-coated epidermal papillae surrounding the stomata. Scale bar: A = 10 μm; B = same magnification.

they are lacking in *Englehardia*, *Oreomunnea*, and *Alfaroa*. Druse crystals are reported here for the first time in *Rhoiptelea*.

10. Rhomboid crystals in leaf palisade: absent (0); present (1). Rhomboid crystals are found only in *Oreomunnea* and *Alfaroa*; both druse and rhomboid crystals are wanting in *Engelhardia* (Stone, 1993; Iljinskaya, 1993).

11. Wood porosity: ring and semi-ring (0); diffuse (1). Genera that are predominantly ring and semi-ring porous may have some species that are diffuse porous. Growth rings are distinct in the temperate species of *Juglans*, but the tropical black walnuts are diffuse porous (Miller, 1976). This is true also of other tropical taxa such as *Engelhardia*, *Oreomunnea*, *Alfaroa*, and *Annamocarya*. *Pterocarya*, however, which is primarily temperate (Manchester, 1987), is also distinctly diffuse porous (Heimsch & Wetmore, 1939).

12. Vessel perforation plates: exclusively scalariform (0); scalariform in part (1); strictly simple (2). *Rhoiptelea* has scalariform perforation plates (Withner, 1941). *Engelhardia*, *Oreomunnea*, and *Alfaroa* have both scalariform and simple perforation plates. All other genera have only simple plates (i.e., the porous vessel perforations of Heimsch & Wetmore, 1939).

13. Mean vessel element length: > 650 μm (0); < 650 μm (1). While there is a great deal of variation in vessel element length within and between samples of a species, a clear trend exists from long elements with scalariform perforation plates and oblique end walls (e.g., *Alfaroa*) to short elements with simple perforation plates and transverse end walls (e.g., *Platycarya*) (Heimsch & Wetmore, 1939). *Engelhardia*, *Oreomunnea*, and *Alfaroa*, have an average vessel element length exceeding 650 μm (673–849 μm: measurements of Miller & Stone: Stone, 1989), whereas *Cyclocarya*, *Pterocarya*, and *Juglans* are intermediate in the 611–524 μm range. *Annamocarya*, *Carya*, and *Platycarya* have elements, on average, that range from 500+ to 432 μm (Leroy, 1953; Stone, 1989). It should be noted here as well that *Platycarya* is the only genus with vascular tracheids (Heimsch & Wetmore, 1939). *Rhoiptelea* has some of the longest vessel elements with an average of 855 μm (Withner, 1941).

14. Vessel pore shape: angular to round (0); strictly round (1). Variation from angular to round is known to occur within four of the five species of *Pterocarya* analyzed by Heimsch and Wetmore (1939); *P. stenoptera*, however, exhibits only angular pores. Exclusively round pores were found in *Annamocarya*, *Carya*, and *Platycarya*. *Rhoiptelea* has mostly angular pores according to Withner (1941).

II. Flowering characters

15. Inflorescences: androgynous (0); strictly unisexual, with pistillate terminal and staminate lateral (1). Individuals with androgynous panicles may also have male and/or female catkins positioned terminally or laterally. Manning (1938) postulated that the inflorescence of the pre-Juglandaceous stock was a highly branched panicle of perfect flow-

ers, and that evolution of the inflorescence within the Juglandaceae proceeded from a terminal androgynous panicle (e.g., *Engelhardia* sect. *Psilocarpeae*), combining central pistillate catkins with several subtending staminate catkins, to separate pistillate and staminate catkins. Further evolution of the pistillate catkin has proceeded with reduction to a few-flowered spike (e.g., *Carya*), whereas the staminate catkin has shifted from a terminal to an axillary position and from a several-catkined panicle to a single catkin (e.g., *Juglans*). Since *Rhoiptelea* has perfect flowers, this character state is not applicable (see #19).

16. Staminate catkins: in clusters or single (0); strictly single (1). As noted above (#15), the single solitary catkin is viewed as the most specialized condition. It is found in *Juglans* and *Pterocarya*, but not in *Cyclocarya*. *Cyclocarya*, with a 3- to 5-catkined panicle, is more similar in this respect to *Annamocarya* (5 to 8) and *Carya* (3). The staminate catkin clusters in *Engelhardia*, *Oreomunnea*, *Alfaroa*, and *Platycarya* tend to be much more variable (1 to 10+) (Manning, 1938, 1978; Stone, 1977).

17. Pistillate catkins: pendent (0); strictly erect at anthesis (1). Genera with species that are predominantly pendent may also have some with erect catkins. Good material is scarce, so these data are incomplete. The pendent pistillate inflorescences are mainly those associated with wind dispersal of seeds. This is certainly true of *Engelhardia*, *Cyclocarya*, and *Pterocarya*. The exact condition in *Oreomunnea* is less certain. Manning (1938, fig. 9) depicted flowering material of *O. pterocarpa* that is certainly pendent, though Stone (1977), based on limited material, noted that the female catkin is erect. Sufficient material has been seen of *O. mexicana*, however, to state with certainty that the pistillate catkin in an androgynous panicle is erect at anthesis (see Stone, 1972, fig. 11). In this connection it is interesting to note that *Alfaroa* with animal-dispersed seeds also has erect pistillate catkins (see Stone, 1977, fig. 6). *Platycarya* has tiny seeds that are wind-dispersed, but the pistillate infructescences are erect, not pendent. This monotypic genus is also unique in the family by exhibiting an entomophily syndrome in which the erect spikes are scented and have stiff showy bracts and very small sticky pollen (Endress, 1986).

18. Pistillate spike: lax (0); condensed (1). Most members have flowers loosely arranged along the inflorescence. The pistillate spike of *Platycarya*, however, looks like a small cone at maturity with long prickly bracts bearing flattened fruits lodged between them (see Endress, 1986).

19. Flowers: bisexual (0); unisexual (1). The flowers of *Rhoiptelea* are arranged in dichasial triplets, with the central flower perfect and fertile and the lateral flowers pistillate, but reduced, sterile, or abortive (Wu & Kubitzki, 1993). Pistillate flowers of the Juglandaceae occasionally have anther-bearing stamens (Manning, 1940), and staminate flowers with an abortive pistil are well documented (Manning, 1948), but these are exceptions and without functional consequence. Su and He (1984: 22), however, suggested that the discovery of bisexual flowers in *Pterocarya stenoptera* "may prove that the unisexual flowers of the present-day Juglandaceae are derived from ancestors with bisexual flowers."

20. Staminate bract: 3-lobed distally (0); 1-lobed distally (1). The nature of the floral envelope in the Juglandaceae has long been a point of discussion. The correlation between the pistillate and staminate flowers of each genus was recognized by de Candolle (1862), Engler (1889), and Nicoloff (1904–1905), leading Manning (1948) to identify the seven parts of the generalized floral envelope as consisting of a bract, two bracteoles, and four sepals (Fig. 4). In those cases where the floral envelope is well developed, there is no difficulty distinguishing between a 3-lobed (viz., *Engelhardia*, *Oreomunnea*, and *Alfaroa*) and a 1-lobed bract (viz., *Pterocarya*, *Cyclocarya*, and *Juglans*). With the reduction in sepals and bracteoles in some genera, the question arises as to whether a 3-lobed structure subtending the stamens is a 3-lobed bract or a 1-lobed bract fused with two lateral bracteoles. This problem resolves itself in the case of *Carya* because the 1-lobed bract is clearly external (anterior) to the two lateral bracteoles (posterior) (Manning, 1948). Initial papers dealing with *Annamocarya* were less clear because Kuang's drawing of the staminate flower (Kuang, 1941: = *Rhamphocarya integrifoliolata*) appears to show the three lobes in one whorl (Manning, 1948). Later studies have concluded, however, that the bract and two bracteoles are in different whorls (Manning & Hjelmqvist, 1951; Stone, pers. obs.). The staminate flower of *Platycarya* is the most difficult to interpret. According to Manning (1978) flowers of this genus lack both bracteoles and sepals, leaving the enlarged bract as the sole component of the floral envelope subtending the exposed stamens (see Endress, 1986). What makes the bract of *Platycarya* potentially difficult to score is the variation in lobing. Samples on the same tree growing in Brooklyn (Manning, 1948: 613) range from clearly 1-lobed to what he referred to as "auricled or angled midway between the base and tip," or, as in the case of a tree growing in Durham, North Carolina (*Stone*

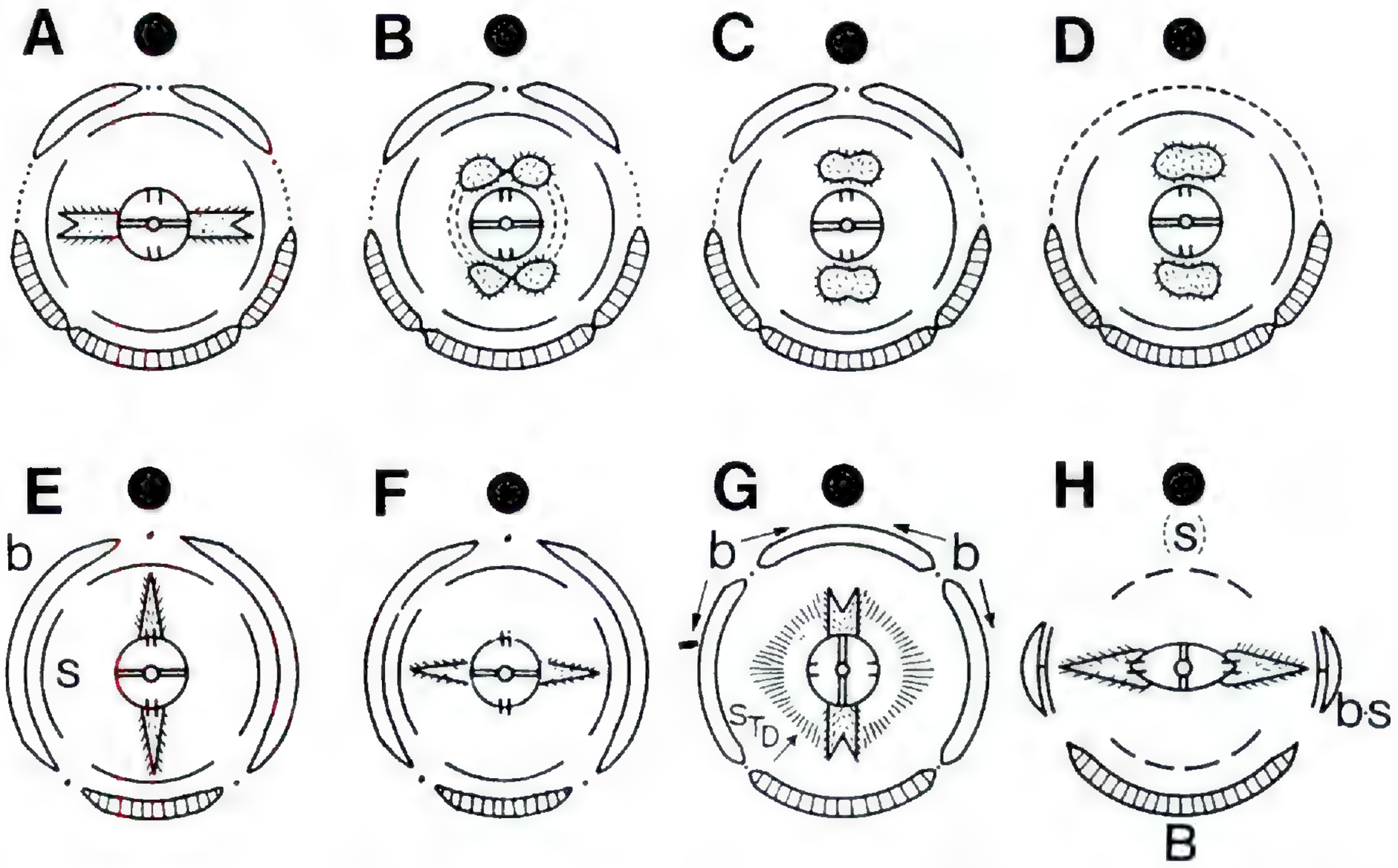


Figure 4. Floral diagrams of pistillate flowers of the Juglandaceae, with the black dots at the top representing the axis of the inflorescence. —A. *Engelhardia* sect. *Engelhardia*. Bract is 3-winged and united at the base to the two fused bracteoles; sepals free; anterior/posterior carpels fuse to form a median oriented primary septum; stigma arms elongate, oriented commissural along plane of primary septum. —B. *Engelhardia* sect. *Psilocarpeae*. Differs from section *Engelhardia* in the carinal orientation of the stigma (split) that is orthogonal to the primary septum. —C. *Oreomunnea*. Same diagram as *E.* sect. *Psilocarpeae*, but the bracteoles form a flap covering the nutlet. —D. *Alfaroa*. The minute 3-lobed bract and imperceptible bracteoles are fused in a small disc at the base of the fruit; the husk of the nut is formed from sepal tissue. —E. *Juglans* and *Pterocarya*. The floral diagrams are similar in most details, including the carinal stigma; however, the bract, bracteoles, and sepals form the husk in *Juglans*, whereas the bracts form the two wings in *Pterocarya*. The primary septum is median and the stigma is carinal. —F. *Cyclocarya*, though having much in common with *Pterocarya*, displays a disc-shaped fruit in which the circular wing develops from adnation of the bract and bracteoles. The primary septum is principally median (see text), and the stigma aligns along the plane of the primary septum in commissural orientation. —G. *Carya* and *Annamocarya*. The husk is formed from the bract and bracteoles and the sepal tissue is modified into a stigmatic disk (uncertain in *Annamocarya*). The primary septum is transverse and the stigma is commissural. —H. *Platycarya*. The bract becomes a stiff pinecone-like scale that subtends a tiny fruit with lateral wings formed from the fusion of the bracteoles and sepals. The primary septum is transverse and the stigma is carinal. Symbols: B = bract; b = bracteole; s = sepal; bs = bracteoles and sepals adnate; STD = stigmatic disk. Figure F is from Manchester (1987), and the others are from Manning (1978).

4071, DUKE), the region is truly flared. The descriptor above is designed to treat *Platycarya* as 1-lobed on the basis that the "flares" are proximal rather than distal as in the truly 3-lobed staminate flowers. At the same time, however, we recognize that the vasculature may suggest the bract of *Platycarya* had a complex origin (see #21).

21. Staminate bract vasculature: 3 or more vascular strands (0); single strand (1). In general, the 3-lobed bracts have 3 or more vascular strands (viz., *Engelhardia*, *Oreomunnea*, and *Alfaroa*) and the 1-lobed bracts have a single strand (viz., *Pterocarya*, *Cyclocarya*, *Juglans*, and *Carya*). The 1-lobed bracts of *Annamocarya* (Fig. 5A) and *Platycarya* (Berquam, 1975; Wing & Hickey, 1984) are exceptions in having 3 or more vascular strands, possibly suggesting a more complex origin of the bract.

22. Male flower bracteoles: present (0); absent (1). The bracteoles are generally present in all genera except *Platycarya* (Manning, 1978), though occasionally some species of *Engelhardia* may be missing one or both (Manning, 1948). The staminate flower of *Platycarya* is virtually naked in lacking both bracteoles and sepals (Manning, 1948; Endress, 1986).

23. Male flower sepals: 4 present (0); 2 present (1); absent (2). Sepals are present in the majority of genera. Sepals are always absent in *Platycarya* and *Annamocarya*, and generally so in *Carya*, though 1 or 2 are occasionally found (Manning, 1948, 1978).

24. Pollen pore distribution: equatorial (0); subequatorial (1); heteropolar (2). Equatorial apertures are the norm in *Platycarya*, *Engelhardia*, *Oreomunnea*, *Alfaroa*, *Cyclocarya*, and *Pterocarya*. The grains of *Carya* and *Annamocarya* have subequatorial pores that are drawn toward the distal pole (outer face of the tetrad), leaving the proximal hemisphere free of apertures (Whitehead, 1965). In

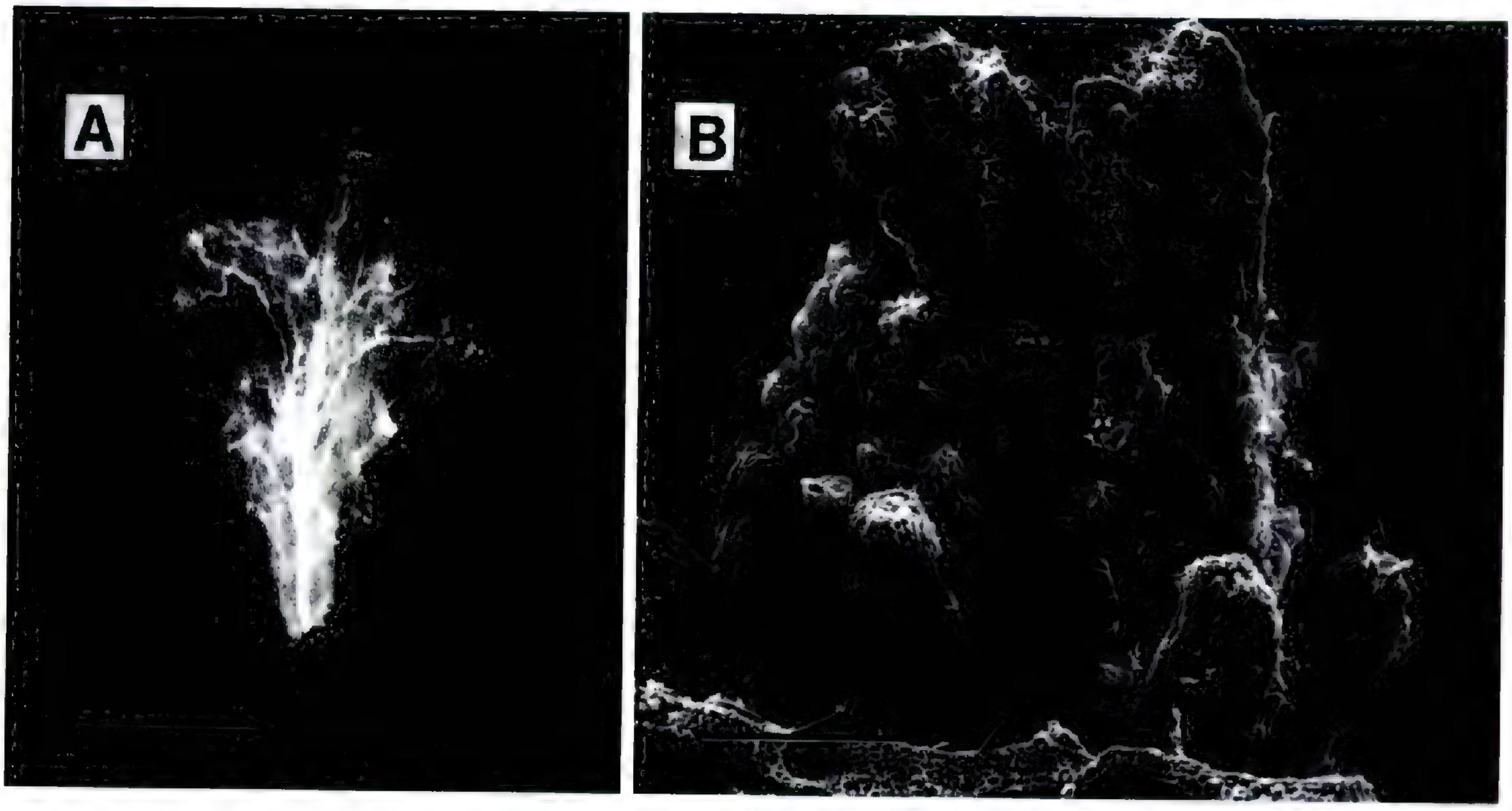


Figure 5. Staminate flower (minus stamens) of *Annamocarya* and the perfect flower (minus stamens) of *Rhoiptelea*. —A. Staminate flower of *Annamocarya* (*Petelot 6376*, A), cleared in sodium hydroxide/chloral hydrate, showing vascular traces in reflected light extending to the terminal bract and two lateral bracteoles. —B. SEM micrograph of perfect flower of *Rhoiptelea* (*Chen 16945*, MO 04193972) with 2 of 4 tepals and the anthers removed to expose 4 of 6 filaments, a scaly ovary topped by two lamelliform stigmas, and 2 tepals in the background. Scale bars: A = 0.5 mm; B = 0.25 mm.

Juglans, however, the grains are heteropolar and multi-aperturate (see #25).

25. Pollen aperture number: predominantly triporate (0); 4 or more (1). The 3-pored grain is characteristic of *Engelhardia*, *Oreomunnea*, *Alfaroa*, *Carya*, and *Annamocarya*. Very rarely 1- to 6-pored grains are found in these genera. *Cyclocarya*, *Pterocarya*, and *Juglans* display (2)4 to 17(37) pores; *Cyclocarya* has grains with 3 and 4 pores, though 4-pored are the most common in extant material; *Pterocarya* commonly has 5- to 7-pored grains, whereas 3 pores are rare; and *Juglans* averages from 6 to 17, though individual grains of the black walnut, *J. nigra*, have been reported to have up to 37 pores (Whitehead, 1963, 1965; Stone & Broome, 1975).

26. Pollen aperture shape: meridionally elongate (0); oblong to circular (1). The meridionally elongate apertures (lolongate of Erdtman, 1966) characterize *Rhoiptelea* (Stone & Broome, 1971), *Engelhardia* sect. *Psilocarpeae*, and *Platycarya* (Whitehead, 1965; Stone & Broome, 1975; Bos & Punt, 1991). *Engelhardia* sect. *Engelhardia* and all of the other genera have oblong to circular pores.

27. Pollen sculpturing: pseudocolpi absent (0); bipolar psuedocolpi present (1). *Platycarya* is unique among angiosperms in having a pair of arcuate pseudocolpi on each polar hemisphere (Whitehead, 1965; Stone & Broome, 1975).

28. Pollen wall: exine not thinning in the proximal pole area (0); exine thinning in the area surrounding the proximal pole (1). According to observations first made by Whitehead (1965), thinning of the exine occurs on the pore-free proximal pole of *Carya* and *Juglans*. The thinning most likely involves reduction of the columella layer (Stone et al., 1964). Manchester and Dilcher (1997) also found this trait in the fossil grains of *Polyptera*.

29. Pollen size: very small, ca. 15 μm (0); 17–26(30) μm, rarely larger (1); > 26 μm (2). The size of individual pollen grains ranges from 11 to nearly 100 μm, with *Platycarya* and *Engelhardia* sect. *Psilocarpeae* having the smallest grains (15 μm); *Engelhardia* sect. *Engelhardia*, *Oreomunnea*, and *Alfaroa* in the intermediate range (17–26 μm); and then a continuum of larger grains, starting with *Cyclocarya* and *Pterocarya* (27–39 μm), *Juglans* (30–49 μm), *Annamocarya* (36–38 μm), and *Carya* (33–66 μm). *Rhoiptelea* grains are triangular in polar view like *Engelhardia roxburghiana* (sect. *Psilocarpeae*) and *Platycarya*, but considerably larger, 23 × 27 μm (Stone & Broome, 1971).

30. Pollen wall structure: nexine moderately thick, equal to ca. one-third thickness of ektosexine (0); nexine extremely thin, less than one-third thickness of ektosexine (1). The nexine of Erdtman (1966), which constitutes the inner footlayer and endexine of Faegri and Iversen (1964), is uniquely

thin in *Engelhardia* sect. *Engelhardia*. All other taxa, including *Rhoiptelea*, *Engelhardia* sect. *Psilocarpeae*, and *Platycarya*, have a relatively thick nexine and ektosexine (= tectum) (Stone & Broome, 1975).

31. Ovary position: superior (0); inferior (1). *Rhoiptelea* has a superior ovary (Fig. 5B), whereas the Juglandaceae have an inferior ovary.

32. Ovary: 2-loculed (0); 1-loculed (1). The ovary of *Rhoiptelea* is bilocular below and unilocular above, with the single anatropous ovule attached to the septum in one of the locules (Wu & Kutbitzki, 1993; Zhang et al., 1994). In the Juglandaceae, the incomplete septum (primary partition) bears the orthotropous ovule at its apex (Manning, 1926), thus bridging both chambers. For this reason the ovary is considered 1-loculed, even though all genera have at least two chambers at the base and the mature fruits may display as many as 8 (see #44).

33. Carpel fusion: median (0); transverse (1). The reference points here are the bract (anterior) and the axis of the catkin (posterior), which are opposite one another (Fig. 4). The two carpels are either anterior-posterior, in which case the primary partition formed from their fusion is median, or lateral to an imaginary line between the bract and the axis, in which case the primary partition is transverse (see Manning, 1940). Manning (1978) recognized *Cyclocarya* as a subgenus of *Pterocarya*, and in doing so did not specifically comment on differences with respect to carpel orientation. He did, however, note that individual flowers are normally median, though they may be oblique or even transverse. Likewise, Manchester (1987) was unable to determine from mature fruits whether the carpels were median or transverse, though he assumed they were median. Our studies of young and maturing flowers of *Cyclocarya* have found that median carpel orientation is the norm (Table 3; Fig. 6A), but oblique and transverse carpels occur 15–20% of the time.

34. Stigmatic disk: absent (0); present (1). While most pistillate flowers have four recognizable sepals (petals are not found in any member of the family), those of *Carya* are modified into a stigmatic disk that is situated at the base of the true stigmas (Manning, 1940). The disk is apparently absent in flowers of *Annamocarya* (Manning & Hjelmqvist, 1951).

35. Stigma orientation: carinal (0); split-carinal (1); or commissural (2). There are normally two stigmatic branches that are oriented orthogonal to the primary septum (carinal) or along the plane of the primary septum (commissural) (Fig. 4). In *Engelhardia* sect. *Psilocarpeae*, where the short stigma is 4-parted, it is also orthogonal to the primary septum (split-carinal) (Manning, 1978). *Cyclocarya* was lumped by Manning with *Pterocarya* in his earlier studies (1940) [though in 1975 he recognized it at the subgeneric level] and, along with *Juglans*, was characterized as having carinal stigmas. Manchester's (1987) examination of both extant and fossil fruits, however, indicated that the style arms of *Cyclocarya* are commissural, rather than carinal. Our studies of young flowers confirm Manchester's observations. The commissural orientation holds, in fact, whether the carpels are median or transverse. In addition, the stigmas of *Engelhardia* sect. *Engelhardia*, *Carya*, and *Annamocarya* are also commissural (Manning, 1978). *Rhoiptelea* is reported to have carinal stigmas (Handel-Mazzetti, 1932; Wu & Kubitzki, 1993).

36. Stigma type: two elongated stylar arms, stigmatic on inner surface (0); two short stylar arms, stigmatic on outer surface (1); obsolete stylar arms, stigma 4-lobed, stigmatic on outer surface (2); two elongated stylar arms, stigmatic on outer surface (3); and obsolete stylar arms, stigmatic papillae on all exposed areas (4). Manning (1940) initially recognized five distinct types of stigmas: papillae on the inner surface of the stigma are found in *Pterocarya*, *Cyclocarya* (Fig. 6B), *Juglans*, and *Platycarya*; papillae on the outer surface characterize *Engelhardia* sect. *Engelhardia*, *Oreomunnea*, *Alfaroa*, and *E.* sect. *Psilocarpeae*, where the first one has long styles, the second and third short styles, and in the fourth they are obsolete; and finally in *Carya* and *Annamocarya* the styles are obsolete and the papillae cover all exposed surfaces. The stigmas of *Rhoiptelea* had not previously been examined from this perspective, but our finding indicates that the style is obsolete, and the two free lamelliform stigmas are stigmatic on all of the exposed surfaces (Fig. 5B). While the stigmas of *Rhoiptelea* are certainly not identical to *Carya*, they both fall in category 4.

37. Ovule type: anatropous (0); orthotropous (1). The embryological study of *Rhoiptelea* by Zhang et al. (1994) confirmed that the ovule is anatropous, crassinucellate, and bitegmic. The derived condition of orthotropous ovules is found in the Juglandaceae, as well as in such dicots as the Piperales, Platanaceae of the Hamamelidales, Myricales, Polygonales, and some genera of Proteaceae and Santalaceae (Stebbins, 1974).

38. Ovule integument: bitegmic (0); unitegmic (1). Reduction or fusion of the inner and outer integuments characteristic of the Juglandaceae is also found in other "Higher" Hamamelididae (see Hufford, 1992; Manos & Steele, 1997). *Rhoiptelea*, on the other hand, is bitegmic.

Figure 6. Young flowers and mature fruits of *Cyclocarya*. —A. Tooth-shaped bract subtending bracteole disk bearing 4 sepals, an inferior ovary, and 2 elongate stigmatic lobes that are aligned with the primary septum, which in this case is median with respect to the axis of the inflorescence and bract (*Stone 4075*, DUKE). —B. Close-up view of the split stigmas to show receptive papillae located on inner stigmatic surface (*Stone 4075*, DUKE). —C. Mature fruit with ragged bracteole wing, the minute tooth-shaped bract intact (bottom center: see arrow), and the central nutlet sanded to expose the transversely oriented primary septum at the equator with two lateral cells of a single locule; further sanding to expose the basal portion of the locule revealed 2, not 4 lobes (*K. Wurdack*, 4/23/98, DUKE). —D. Cleared and stained circular wing of a mature fruit (*Wilson 452*, AA) showing a loose arrangement of terminating veins along the bracteole margins and two parallel vascular strands entering the tooth-like bract. Scale bars: A = 1 mm; B = 0.5 mm; C = 5 mm; D = 0.33 mm.

III. Fruiting characters

39. Pistillate bract: free of fruit (0); attached to the fruit (1). In the case of *Platycarya* the stiff pointed bracts remain attached to the axis of the infructescence while the small fruits are shaken from the "cone" by wind.

40. Pistillate bract: large and conspicuous (0); small or inconspicuous and fused to other floral parts (1). The large bracts of *Engelhardia* sect. *Engelhardia*, *E.* sect. *Psilocarpae*, and *Oreomunnea* are associated with wind dispersal of the fruits.

41. Pistillate bract: trilobed (0); unlobed (1). The large-bract taxa (#40) are also trilobed, as is *Alfaroa*, which has a small, inconspicuous bract that adheres to the base of the nutlet (see Stone, 1997).

42. Pistillate bracteoles: bracteoles rudimentary or absent, the ovary and stigmas exposed (0); bracteoles 2, posterior, forming a moderately high lobe obscuring the ovary but leaving the stigmas exposed, loosely to tightly adhering to the ovary (1); bracteoles 2, posterior, forming a high lobe obscuring the ovary and stigmas, fused to the ovary about halfway up (2); bracteoles 2, lateral, fused to the ovary about halfway up (3); bracteoles 2, lateral, fused to top of ovary (4); and bracteoles 3 to 5, lateral and posterior, fused to the top of the ovary (5). In the case of *Engelhardia* sect. *Psilocarpeae* and *Alfaroa* the bracteolar lobe (prophyllum) is rudimentary or absent and the pistil is fully exposed to the elements. In *Engelhardia* sect. *Engelhardia* the paired bracteoles are fused to the lower half of the ovary on the adaxial side to form a moderately high lobe that protects the ovary but leaves the stigmas exposed. The bracteolar lobe of *Oreomunnea* extends far above the stigmas to encase the entire pistil (Stone, 1972). In those cases where the bracteoles are fused on the lateral flanks from the middle of the ovary or above, they form the 2-winged nutlet in *Pterocarya*, the circular wing of *Cyclocarya*, the thick husk of *Juglans*, or the elm-like fruit of *Platycarya* with minute lateral wings. In *Carya* and *Annamocarya* the bracteoles are interpreted by Manning (1940, 1978) as being 2, in which case they split into 3 in *Carya* and 5 in *Annamocarya*. In this latter case, the bracteole numbers are unique for the family, positioned in both a posterior and lateral fashion, and fused to the top of the ovary to participate in the formation of a thick husk.

43. Fruit type: simple fruit (0); accessory fruit with wings—wings formed by 3-lobed bract (1); wings formed by bract and 2 bracteoles (2); wings formed by 2 bracteoles (3); wings formed by 2 lateral sepals and 2 bracteoles (4); accessory fruit without wings—husk formed by 4 sepals (5); husk formed by bract, 2 bracteoles and 4 sepals (6); husk formed by bract and bracteoles (7). While the developmental pathways leading to the production of fruits in *Rhoiptelea* and the Juglandaceae are varied (see Discussion), the modes of fruit dispersal are of two types: wind and animal. The small (5–7 mm), flattened, elm-like fruits of the monotypic *Rhoiptelea* are derived solely from the ovary, and hence lack accessory floral components in formation of the wind-dispersal unit. The nature and extent of wing development in the Juglandaceae, however, varies greatly between genera, and is in fact one of the principal features used to recognize extant and fossil fruits (Manchester, 1987). Those genera with pendent inflorescences and fruits with well-developed wings such as *Engelhardia*, *Oreomunnea*, *Pterocarya*, and *Cyclocarya* are clearly associated with wind dispersal (Stone, 1973). *Platycarya*, on the other hand, has condensed infructescences that are persistent into the next flowering season and remain upright on the branches, with the slow release of the small winged-seeds as the cones are shaken by the wind. Animal-dispersed fruits lack wings, are relatively large, and are covered with a husk (Stone, 1989). In the case of *Alfaroa* the husk is thin and persistent; in most species of *Juglans* it is indehiscent, though that of *J. regia* sect. *Juglans* is irregularly dehiscent into 4 valves (Manning, 1978); and in *Carya* and *Annamocarya* the husk splits more or less completely into 4 to 6 valves and the nut becomes free from the husk at maturity (Manning & Hjelmqvist, 1951; Manning, 1978).

44. Fruit chambers at base: 2 (0); 2 to 4 (1); 8 (2). The chambers are the product of primary, secondary, and occasionally tertiary partitions that form at the base of the fruit and extend to varying degrees toward the top. There are consistent generic and sectional differences within the 2- to 4-chambered fruit types (Manning, 1978). The fruits of *Platycarya*, *Juglans* sect. *Cardiocaryon*, and *J.* sect. *Trachycaryon* are 2-chambered without any signs of a secondary partition. *Annamocarya* is basically 2-chambered at the base but it does have a weakly developed secondary partition represented by a pair of basal buttresses that extend out from the primary partition (Manning & Hjelmqvist, 1951; Leroy, 1955). *Engelhardia*, *Pterocarya*, *Juglans* sects. *Juglans* and *Rhysocaryon*, and *Carya* are distinctly 4-chambered. Though *Cyclocarya* is noted by Manning (1978: 1075) to have "a nutlet 2-celled at the base," as we found in a limited sample (see Fig. 6C), Manchester has determined that there is a secondary septum in at least some extant fruits and that "well preserved fossil *Cyclocarya brownii* fruits . . . always show 4 distinct locule

lobes [and] a well developed secondary partition" (Manchester, pers. comm.). The 8-chambered fruits characteristic of *Alfaroa* and *Oreomunnea* result from the addition of tertiary septa (Stone, 1977).

45. Nutshell sclerenchyma: principally fibers (0); principally isodiameteric sclereids (1). This is a character discovered by Manchester and first made available to Smith and Doyle (1995).

IV. Seed and seedling characters

46. Cotyledons: epigeous (0); hypogeous (1). Epigeal germination is characteristic of most of the wind-borne fruits, including *Platycarya, Engelhardia,* and *Pterocarya. Cyclocarya,* however, is reported to have hypogeal germination (Iljinskaya, 1990). Likewise, *Oreomunnea* is a wind-dispersed species, but it has hypogeal germination like the animal-dispersed nuts of *Alfaroa.* Hickories (*Carya* and *Annamocarya*) and walnuts (*Juglans*) also have hypogeal germination (Stone, 1973; Grauke, pers. comm.). And to our surprise, the wind-borne seeds of *Rhoiptelea* have hypogeal germination as well (Stone, pers. obs., *Stone 4110,* DUKE).

47. Cotyledonary node: one gap (0); 2+ gaps (1). A single gap is associated with the wind-borne fruits with epigeous cotyledons of *Pterocarya* (and probably *Cyclocarya*), *Platycarya,* and *Engelhardia,* the wind-borne fruits of *Oreomunnea* with hypogeous cotyledons, and the animal-dispersed fruits of *Alfaroa* with hypogeous cotyledons. The large nuts of *Juglans,* with hypogeous cotyledons, have 2 gaps, whereas those of *Carya* (and probably *Annamocarya*) have 3 or more gaps (Conde & Stone, 1970). *Cyclocarya,* like *Oreomunnea,* has wind-dispersed seeds that display hypogeal germination. Since the cotyledonary node has not been examined, both the gaps and traces (character 48) are treated as unknowns (Table 3).

48. Cotyledonary node: two traces (0); 3+ traces (1). The 2-trace condition is found in those taxa having wind-borne fruits and epigeous cotyledons, including *Pterocarya, Platycarya,* and *Engelhardia,* as well as in the animal-dispersed fruits of *Juglans* with hypogeous cotyledons (Conde & Stone, 1970; Stone, 1970). The 3-trace condition is characteristic of the animal-dispersed hypogeal cotyledons of *Alfaroa* and its sister genus *Oreomunnea* with wind dispersal. The higher trace numbers occur in *Carya,* and probably as well in *Annamocarya.* What may appear to be a bewildering array of nodal diversity is confined to those groups with hypogeal germination. All taxa with epigeal germination have the conservative 1-gap, 2-trace node (Eames, 1961). In those taxa with epigeal germination the petiole of the cotyledons displays a pair of lateral strands and a central one that splits near the gap to send one half to each of the laterals, thus entering the node as a bundle of 2 strands or traces. In the case of the hypogeous *Alfaroa* and *Oreomunnea,* the central strand does not split so that 3 sets of traces enter the node through a single gap. The hypogeous condition of *Carya* and *Juglans* is more complex with the formation of additional gaps and the vascularization coming from the separation of the lateral strands, splitting of the central strand, or even the formation of accessory strands in the 5 or more-gap nodes.

49. Seed oils: linolenic > linoleic/palmitic low (0); linolenic > linoleic/palmitic high (1); palmitic high/linoleic > oleic (2); linoleic > oleic/palmitic low (3); oleic > linoleic/palmitic low (4). The lipids of seeds in particular are generally known to characterize some plant families, genera, and even species (Hilditch & Williams, 1964). Five fatty acids have been identified in the Juglandales (Stone et al., 1965, 1969; Stone, 1989; Jiang & Zhou, 1990; Zhou & Jiang, 1990), two of which are saturated (viz., palmitic and stearic) and three are unsaturated (viz., oleic, linoleic, and linolenic). Only stearic acid is essentially uniform throughout with concentrations in individual samples varying from 0 to 16%, but the species' average is well below 5%. Each of the other four fatty acids characterize groups of taxa (Table 4). *Rhoiptelea, Platycarya, Pterocarya,* and *Cyclocarya,* for example, have high concentrations of linolenic, somewhat lower linoleic, and low concentrations of palmitic acid (CB/low pattern); *Engelhardia,* on the other hand, has slightly more linolenic than linoleic on the average, and high palmitic (CB/high pattern); *Oreomunnea, Alfaroa,* and *Annamocarya* are characterized by more linoleic than oleic, and high palmitic (BA/high pattern); *Juglans* is autapomorphic for high concentrations of linoleic, considerably less oleic, and low palmitic (BA/low pattern); and *Carya* has uniquely high concentrations of oleic, considerably less linoleic, and low palmitic (AB/low pattern).

V. Chromosomes

50. Base chromosome number: $x = 16$ (0); $x = 15$ (1); $x = 28$ (2). While the base number of chromosomes for the family is $x = 16$, tetraploidy occurs in *Carya* and aneuploidy is found in *Platycarya* and *Cyclocarya* (see Discussion). Although chromosome number is not phylogenetically informative in the sense of phylogenetic placement, we include it in the analysis to provide autapomorphic support to aid in the recognition of genera.

MOLECULAR CHARACTER ANALYSIS

A total of 206 potentially informative characters was obtained from sequencing two cpDNA intergenic spacers and the ITS region of nuclear ribosomal DNA (Table 5). The final alignments of the cpDNA spacers and ITS region suggested the presence of insertion and/or deletion events (indels or gaps) of limited phylogenetic value. Gaps in particular taxa were treated as missing data in subsequent phylogenetic analyses.

Pairwise comparisons based on the cpDNA sequences showed that raw sequence divergence was low with all values falling below 5.0%. Sequences of the nuclear ITS region showed appreciably more sequence divergence, with the highest values obtained in pairwise comparisons between ingroup and outgroup (15.7–18.4%) and somewhat lower values (10.0–15.0%) between ingroup taxa representing the two major groups within the family. For Juglandaceae, the nuclear ITS region appears to be evolving about 3–5 times faster than the noncoding cpDNA spacers examined here.

PHYLOGENETIC ANALYSES

Separate analysis of each data set yielded minimum length trees generally identifying two basic clades within Juglandaceae, the Engelhardioideae (*Engelhardia*, *Oreomunnea*, and *Alfaroa*), and the remaining genera (Fig. 8). Morphological/chemical data generally showed lower resolution because of character conflict, although lack of infrageneric resolution is due primarily to invariant character states (Fig. 8A; Tables 3, 5). Chloroplast DNA sequences produced trees with the least homoplasy, whereas trees derived from ITS sequences had more homoplasy but more resolution (Fig. 8B, C; Table 5). Cladistic relationships were mostly congruent with moderate to high bootstrap support for most of the major clades. Although topological congruence predominated among the trees generated by separate analysis (Fig. 8), interspecific discordance between ITS and cpDNA trees was observed within *Alfaroa* (Fig. 8B, C). The lack of support for the monophyly of *Juglans* based on ITS data also was notable. Combining data sets generally resulted in more resolution and fewer trees of equal length (Table 5). The four most parsimonious trees derived from combined analysis of the three data sets differed only in the resolution within the genus *Carya*. In these trees morphological data contributed approximately 15% of the total synapomorphy relative to cpDNA (23%) and ITS (62%).

Trees based on the combination of all data were 13 steps longer than additive lengths of the three data sets indicating relatively low levels of character incongruence ($I_{MF} = 0.025$). In pairwise comparisons values for the lowest estimate I_{MF} were obtained in the combined analysis of Morph/chem + cpDNA, which was one step longer than the additive lengths of the data sets analyzed separately (Table 5). The results of the incongruence length difference test between and among data sets indicated some degree of incongruence among data sets, especially between cpDNA and ITS (Table 5).

CHARACTER STATE RECONSTRUCTIONS: CLADE AND GENUS DIAGNOSES

According to the trees based on combined analysis, most of the recognized genera and clades within the family were supported by a combination of molecular and morphological apomorphies (Figs. 8, 9). Because topological differences among the four most parsimonious trees were restricted to the New World clade of *Carya*, morphological characters with alternative most parsimonious reconstructions could be evaluated using a single tree. Nine of the 38 phylogenetically informative morphological characters showed more reasonable optimizations under *deltran*, and parallel changes were favored for reasons related to generic delimitation and biologically realistic patterns of character state transformation in the taxa under study. Using a combination of reconstructions, unique character state changes could be found to support most of the clades and genera resolved in the combined analysis (Fig. 9). Exceptions included the *Pterocarya* + *Cyclocarya* clade and the genus *Annamocarya*, both of which were defined consistently by only homoplasious character states.

The Engelhardioideae clade (Fig. 9) is characterized by even-pinnate leaves (character 6-state 1), the absence of druse crystals (9-1), and the possession of both scalariform and simple vessel perforation plates (12-1). The presence of accessory fruits with a 3-lobed wing (43-1) also defined the clade, except in *Alfaroa* where the condition is transformed uniquely to form a fruit husk (43-5). The two species of *Engelhardia* examined lack synapomorphies. *Engelhardia spicata* is defined by a thin nexine (30-1), stigmas on outer surface of the 2 elongated stylar arms (36-3), and pistillate bracteoles adhering to the ovary (42-1). *Engelhardia roxburghiana* is defined by male flowers with 2 sepals (23-1) and stigmas on the outer surface of the reduced 4-lobed stylar arms (36-2). Sister taxa *Oreomunnea* and *Alfaroa* share several unique character states: opposite leaves (5-1); papillae on the lower surface of leaflets (8-1); rhomboid crystals in

Table 4. Fatty acid composition of Rhoipteleaceae and Juglandaceae seeds.

Taxon	Source*	No. samples	% Palmitic (saturated)	% Stearic (saturated)	% Oleic (unsat.) A	% Linoleic (unsat.) B	% Linolenic (unsat.) C	Unsat. 1st & 2nd highest %	Palmitic low or high**
Rhoiptelea *chiliantha* Diels & Handel-Mazzetti	*J & Z*	**1**	**8**	**2**	**9**	**30**	**51**	**CB**	**low**
Engelhardia *roxburghiana* Wallich		**AVE.**	**23**	**2**	**14**	**28**	**34**	**CB**	**high (or low)**
	S	1	38	3	16	26	18		
	J & Z	1	8	1	11	29	50		
Oreomunnea		**AVE.**	**48**	**5**	**22**	**25**	**1**	**BA**	**high**
O. mexicana (Standley) Leroy	*S*	3	59	3	18	18	2		
O. pterocarpa Oersted	*S*	1	37	6	25	32	tr***		
Alfaroa		**AVE.**	**31**	**3**	**19**	**44**	**5**	**BA**	**high**
A. costaricensis Standley	*S*	1	24	3	12	59	2		
A. guanacastensis Stone	*S*	3	32	3	22	38	6		
A. guatemalensis (Standley) L. O. Williams	*S*	1	26	2	16	49	7		
A. manningii Léon		2	41	2	25	31	3		
Platycarya *strobilaceae* Siebold & Zuccarini		**AVE.**	**11**	**2**	**6**	**26**	**56**	**CB**	**low**
	S	1	11	1	4	28	55		
	J & Z	1	10	2	8	24	56		
Pterocarya *stenoptera* C. DC.		**AVE.**	**9**	**3**	**10**	**31**	**50**	**CB**	**low**
	S	1	7	1	8	32	53		
	J & Z	1	10	4	11	29	46		
Cyclocarya *paliurus* (Batalin) Iljinskaya	*J & Z*	**1**	**8**	**2**	**15**	**32**	**44**	CB	low
Juglans		**AVE.**	**4**	**1**	**21**	**69**	**4**	**BA**	**low**
J.* sect. *Juglans, *J. regia* L.		**AVE.**	**7**	**2**	**17**	**65**	**11**		
	S	1	7	tr	14	68	11		
	J & Z	1	6	3	19	62	10		
J.* sect. *Rhysocaryon		**AVE.**	**5**	**2**	**27**	**64**	**3**		
J. australis Grisebach	*S*	1	7	3	27	61	2		
J. boliviana (C. DC.) Dode	*S*	1	4	0	22	70	4		
J. hirsuta Manning	*S*	5	3	0	20	75	2		
J. microcarpa Berlandier	*S*	1	4	2	17	70	7		
J. mollis Engelmann ex Hemsley	*S*	1	5	tr	49	46	0		
J. neotropica Diels	*S*	1	5	3	39	50	3		
J. nigra L.	*S*	4	3	2	23	69	3		
J. olanchana Standley & L. O. Williams	*S*	1	9	1	18	73	tr		

Table 4. Continued.

Taxon	Source*	No. samples	% Palmitic (saturated)	% Stearic (saturated)	% Oleic (unsat.) A	% Linoleic (unsat.) B	% Linolenic (unsat.) C	Unsat. 1st & 2nd highest %	Palmitic low or high**
J.* sect. *Cardiocaryon		**AVE.**	**2**	**0**	**16**	**77**	**6**		
J. ailantifolia Carrière	*S*	1	2	0	18	74	7		
J. mandshurica Maximowicz	*S*	1	2	tr	14	79	5		
***J.* sect. *Trachycaryon*, *J. cinerea* L.**	*S*	**2**	**2**	**0**	**13**	**71**	**15**		
Carya		**AVE.**	**7**	**2**	**65**	**20**	**1**	**AB**	**low**
***C.* sect. *Sinocarya*, *C. cathayensis* Sargent**	***J & Z***	***1***	**3**	**1**	**79**	**14**	**2**		
C.* sect. *Apocarya		***AVE.***	**10**	**2**	**65**	**23**	**1**		
C. aquatica (F. Michaux) Nuttall	*S*	5	9	2	53	35	1		
C. cordiformis (Wangenheim) K. Koch	*S*	14	9	3	73	14	2		
C. illinoinensis (Wangenheim) K. Koch	*S*	7	6	2	72	19	1		
C. palmeri Manning	*S*	4	15	2	61	23	0		
C.* sect. *Carya		**AVE.**	**9**	**2**	**51**	**38**	**0**		
C. floridana Sargent	*S*	4	8	3	50	40	0		
C. glabra (Miller) Sweet	*S*	19	8	2	47	43	0		
C. glabra (*ovalis* type)	*S*	6	10	3	53	35	0		
C. laciniosa (F. Michaux) Loudon	*S*	6	8	2	56	34	1		
C. myristiciformis (F. Michaux) Nuttall	*S*	8	9	3	54	34	0		
C. ovata (Miller) K. Koch *var. ovata*	*S*	7	11	2	55	32	0		
C. ovata var. carolinae-septentrionalis (Ashe) Reveal****	*S*	5	9	2	53	37	0		
C. pallida (Ashe) Engler & Graebner	*S*	4	10	2	46	42	0		
C. texana Buckley	*S*	31	8	2	50	39	1		
C. tomentosa (Poiret) Nuttall	*S*	34	8	2	48	42	1		
Annamocarya sinensis (Dode) Leroy	***J & Z***	**1**	**30**	**tr**	**19**	**41**	**8**	**BA**	**high**

* *S* = D. E. Stone, unpublished; *J & Z* = Jiang & Zhou (1990).

** low = <20% (2–15); high = >20% (24–59).

*** tr = trace amount.

**** According to the Demoulin Rule (Reveal, 1983), *Carya ovata* var. *carolinae-septentrionalis* takes precedence over *C. ovata* var. *australis* (Ashe) Little (Stone, 1997).

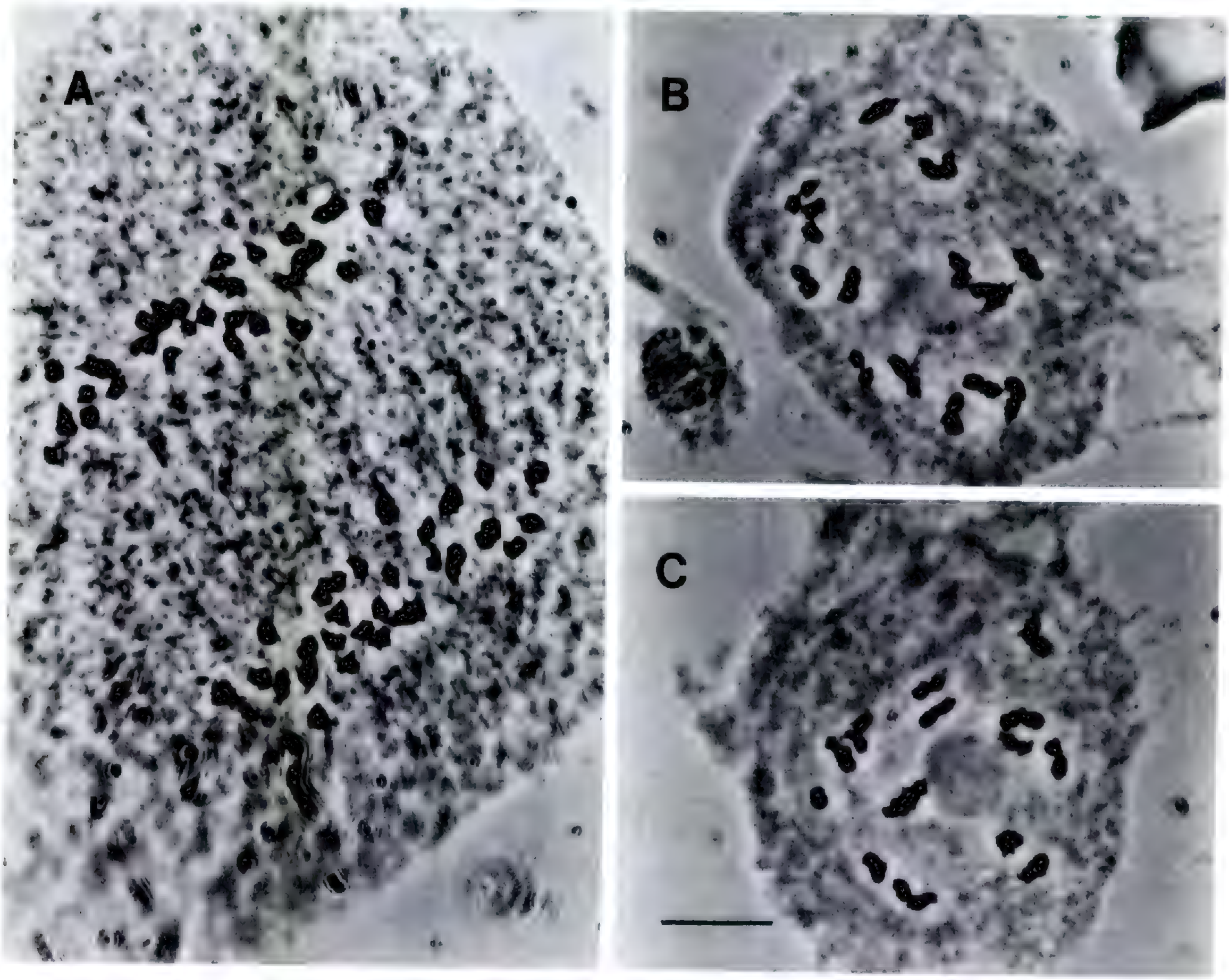


Figure 7. Meiotic chromosomes in pollen mother cells of *Cyclocarya* and *Platycarya* photographed under phase contrast. —A. Micrograph of *Cyclocarya* (*Stone 4075*, DUKE) showing 28 chromosomes moving to each pole in anaphase I. —B. Micrograph of *Platycarya* with 15 bivalents (the prevailing number) in diakinesis. —C. Micrograph of *Platycarya* with 14 bivalents in diakinesis. Questions have been raised about the possibility that the large chromosomal unit attached to the left side of the nucleolus might in fact represent two bivalents. To the best of my (DES) microscopic observation, this unit is a single bivalent. Scale bar: A, B, and C = 10 μm.

Table 5. Comparisons of the data sets generated in this study and results of parsimony analysis on single and combined data sets. CI = consistency index (excluding autapomorphies); RI = retention index; I_{MF} = Index of Mickevich & Farris (1981); ILD = Incongruence length test (Farris et al. 1994).

Data set no.	Data set title	Total no. characters	No. informative characters	No. trees/tree length	CI/RI	Nodes supported in consensus	Sum of tree lengths/I_{MF}/ILD *P* value
1.	Morphology/Chemical	50	38	6/88	0.68/0.91	6	—
2.	cpDNA	1257	56	2/70	0.87/0.97	16	—
3.	ITS region	678	150	2/338	0.60/0.83	18	—
1 + 2	Morph/Chem + cpDNA	1307	94	8/159	0.76/0.93	21	158/0.006/0.95
1 + 3	Morph/Chem + ITS	727	188	1/430	0.62/0.85	22	426/0.009/0.48
2 + 3	cpDNA + ITS	1934	206	8/419	0.63/0.86	18	408/0.026/0.01
1 + 2 + 3	All data combined	1984	244	4/509	0.64/0.87	20	496/0.025/0.04

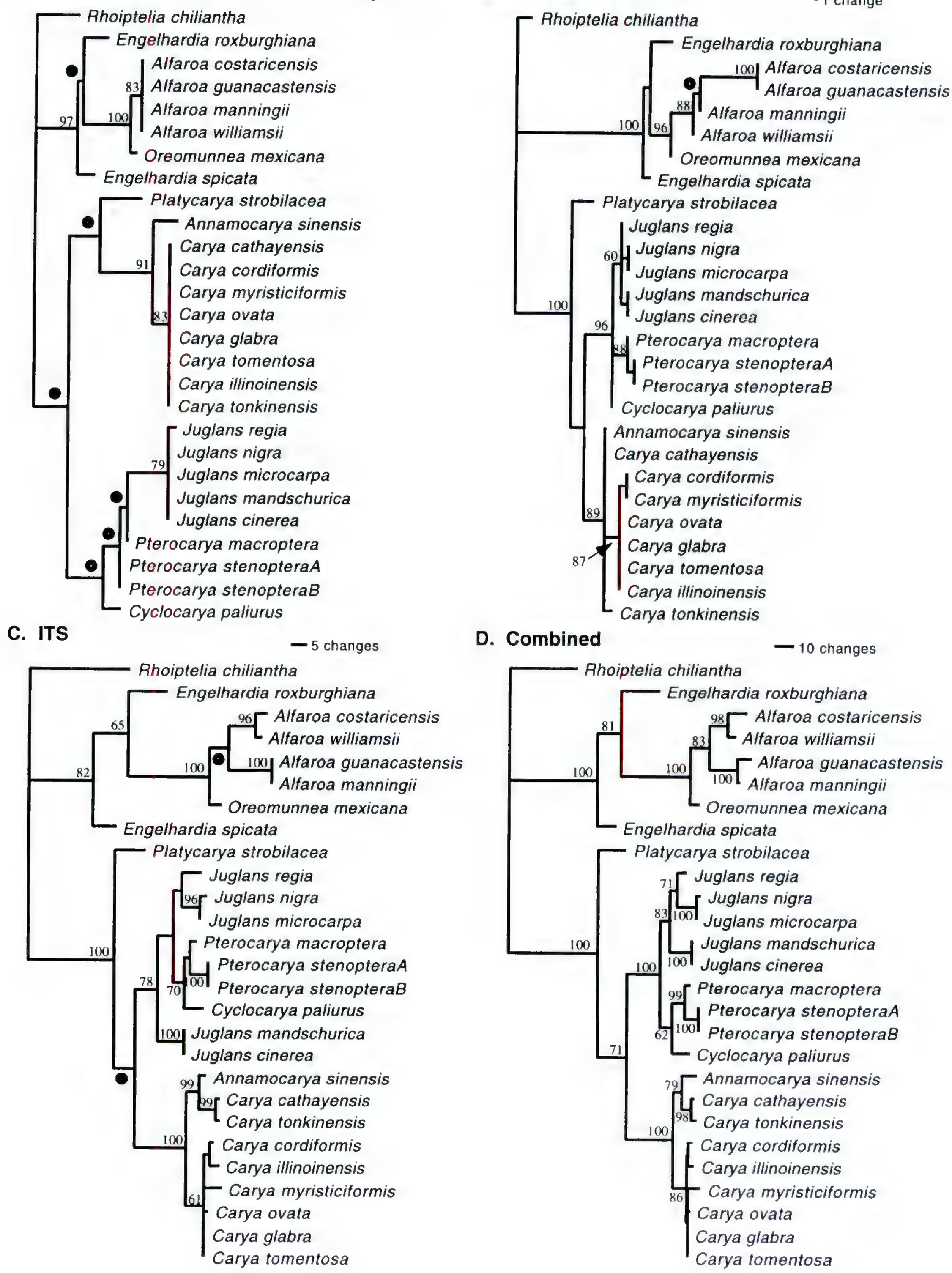


Figure 8. Phylograms of the Juglandaceae. —A. One of 8 shortest trees based on morphology/chemistry. —B. One of 2 shortest trees based on cpDNA sequences. —C. One of 2 shortest trees based on ITS sequences. —D. One of 4 shortest trees based on combined analysis. Percentage of 1000 bootstrap replications is given for nodes with values > 50%. Nodes not supported in the strict consensus are indicated with a solid circle.

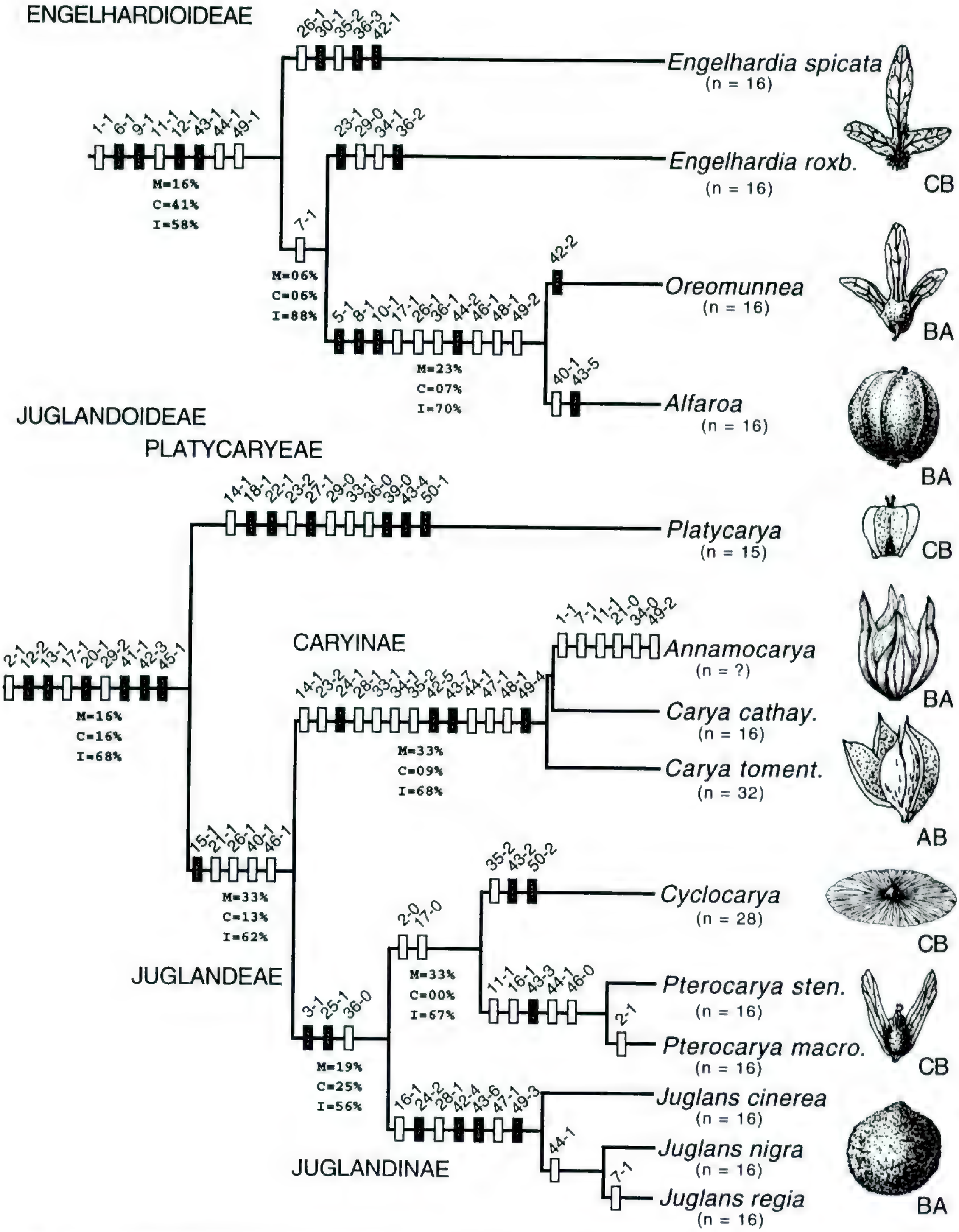


Figure 9. Cladogram of the Juglandaceae based on the strict consensus of 4 trees produced by combined analysis of three data sets: Engelhardioideae; Juglandoideae. Character numbers and states are indicated with hash marks (see Table 3 and Results). Unique and homoplasious character states are indicated with solid and open hash marks, respectively. The percentage of apomorphy contributed by individual data set (M = morphology/chemistry; C = cpDNA; I = ITS) relative to total branch length is provided below the branches uniting genera. Under each fruit type abbreviations for lipid content are provided (see text and Table 4): CB = high linolenic and linoleic fatty acids, low palmitic; BA = high linoleic and oleic, high palmitic; AB = high oleic and linoleic, low palmitic.

leaf palisade (10-1); and 8-chambered fruit (44-2). *Oreomunnea* is further defined by the bracteolar lobe that envelops the pistil (42-2).

The Juglandoideae clade (Fig. 9) is defined by simple vessel perforation plates (12-2), vessel elements less than 650 μm in length (13-1), 1-lobed staminate bracts (20-1), unlobed pistillate bracts (41-1), and nutshell composed of sclereids rather than fibers (45-1). The lateral position and fusion of the 2 pistillate bracteoles to the ovary (42-3) is also unique to the clade, except in the cases of *Carya* and *Annamocarya* (42-5), where 2 bracteoles split into 3 or more segments and have a posterior as well as lateral position. *Platycarya* is highly autapomorphic as indicated by condensed pistillate spikes (18-1), absence of staminate bracteoles (22-1), pollen with pseudocolpi (27-1), a free pistillate bract (39-0), fruit wings formed by 2 lateral sepals and 2 bracteoles (43-4), and a unique aneuploid chromosome number of $x = 15$ (50-1). The remaining genera of the clade are united by strictly unisexual inflorescences in the following positional arrangement: pistillate terminal and staminate lateral (15-1). *Carya* (including *Annamocarya*) is defined by subequatorial pollen pores (24-1), 2 lateral pistillate bracteoles fused to the top of the ovary (42-5), the unique derivation of a fruit husk formed, in part, by associated pistillate bracteoles (43-7), and the following unique fatty acid proportions in the seed: palmitic low/oleic > linoleic (49-4). The genera *Cyclocarya*, *Pterocarya*, and *Juglans* are defined by a chambered pith (3-1) and pollen with 4 or more apertures (25-1). *Cyclocarya* is defined by having fruit wings formed by the fusion of the bract and 2 bracteoles (43-2) and the unique aneuploid chromosome number of $x = 28$ (50-2). *Pterocarya* is defined by having fruit wings formed by 2 bracteoles (43-3). *Juglans* is characterized by heteropolar pollen pores (24-2), 2 lateral bracteoles fused to the top of the ovary (42-4), a fruit husk formed by the combination of bract, 2 bracteoles, and 4 sepals (43-6), and unique fatty acid proportions in the seed, namely: linoleic > oleic/palmitic low (49-3).

Discussion

Phylogeny and Multiple Lines of Evidence

Cladistic analyses of DNA sequence and morphological data of the taxa surveyed in this study support two major clades within Juglandaceae, results that are consistent with the most recent cladistic analysis based on cpDNA restriction sites and morphology (Smith & Doyle, 1995). Both studies contradict an earlier morphological cladistic study of the family by Wing and Hickey (1984), which suggested *Platycarya* was sister to the Engelhardieae on the basis of synapomorphic foliar characters: basilaminar region with dense hair patches; fewer than 10% of the areoles with free-ending veinlets; well developed intersecondary veins; and distinction between fourth- and higher-order venation weak or absent (none of these characters were analyzed in this study). Although Smith and Doyle (1995) limited their study to a single exemplar from seven of the ten recognized genera and employed distant outgroups (*Quercus*, *Myrica*, and *Betula*), they essentially found the same result as presented here (Figs. 8D, 9). The major emphasis of our contribution is a broad-based systematic characterization of the family using three independent data sets derived from a diverse taxonomic sample. Our study is distinctive in that we have obtained sequence data, most importantly from a rapidly evolving region of nuclear ribosomal data (ITS), and in addition employed the relevant outgroup, *Rhoiptelea*, for rooting trees and assessing character polarity as accurately as possible.

Combining evidence from the three independent sources of character data was essential in deriving the most well supported phylogenetic hypothesis for the taxa under study (Figs. 8D, 9). This is best exemplified by comparing the resolution and support for particular nodes in separate analyses (Fig. 8; Table 5). With respect to data decisiveness, the combination of morphological and ITS data produced only one tree and resolved the greatest number of nodes. However, an increase in bootstrap support resulted upon the addition of the cpDNA data, in spite of the decrease in the number of nodes resolved within New World *Carya*. The most striking aspect of the effects of data combination within the family involves particular infrageneric cases. The relatively strong hypothesis for the paraphyly of *Engelhardia* (Fig. 8D) provides an instructive example of the increased explanatory power of combined analysis (e.g., Kluge, 1989; Kluge & Wolfe, 1993; Olmstead & Sweere, 1994; Allard & Carpenter, 1996; Nixon & Carpenter, 1996; DeSalle & Brower, 1997; Soltis et al., 1998). According to the trees derived from combining morphological and cpDNA data (not shown), a paraphyletic *Engelhardia* is supported in the consensus tree, but only with a bootstrap value of 69%, whereas the addition of ITS data is critical to bolstering the case for paraphyly (81%). Similarly, the branch supporting the monophyly of taxa traditionally placed in the tribes Juglandeae (*Juglans*, *Pterocarya*, and *Cyclocarya*) and Caryinae (*Carya* and *Annamocarya*) is found only in the cpDNA consensus tree, but without much relative support (Fig. 8B).

In contrast, the combined tree shows the emergence of relatively strong phylogenetic signal to resolve this node with additional confidence (Fig. 8D).

Another important aspect of obtaining multiple data sets is illustrated by the resolution of the species of *Juglans* (Fig. 8). According to the ITS data (Fig. 8C), classically recognized sections within *Juglans* form two monophyletic groups, with species of section *Rhysocaryon* (*J. nigra* and *J. microcarpa*) and *J. regia* (sect. *Juglans*) resolved as more closely related to the clade containing *Pterocarya* and *Cyclocarya* than to other species of *Juglans*. While the support for this arrangement is modest, combined analysis of ITS + cpDNA data sets is also influenced strongly by ITS, such that the monophyly of *Juglans* is not supported in the consensus trees (not shown). Clearly, unequal rates of sequence divergence or perhaps phylogenetic sorting of ITS alleles has produced conflicting signal at odds with the support for the monophyly of *Juglans* based on morphology, cpDNA, and combined analysis (Fig. 8).

Previous phylogenetic studies of *Juglans* generally supported two groups of species: section *Rhysocaryon* (black walnuts) and section *Cardiocaryon* (butternuts, including sect. *Trachycaryon*); however, the placement of *J. regia* (sect. *Juglans*) is problematic (Fjellstrom & Parfitt, 1994, 1995; Stanford et al., 2000). The most recent study based on broad sampling and sequence data from the ITS region and cpDNA gene *mat*K (Stanford et al., 2000) placed *J. regia* with the butternuts. Our analysis instead supports a sister group relationship with the black walnuts, thus challenging the notion that the black walnuts in the broad sense are strictly New World in distribution (Stanford et al., 2000). Therefore, in addition to the well-supported disjunction within the butternuts, where all but *J. cinerea* occur in Asia, our results suggest a second biogeographic disjunction within *Juglans*. Clarifying the position of *J. regia* with additional material is essential to interpreting the biogeographical history of the genus.

In considering the weight of independent lines of evidence, we have attempted to quantify the relative contribution of each data set to the branches uniting two or more genera in the combined analysis (Fig. 9). Although this measure is roughly a function of the number of phylogenetically informative characters in each data set (see Table 5), several observations point to the contribution of morphological data in providing the best supported hypothesis of the evolutionary history of Juglandaceae. For every internal branch resolved, morphological support is often higher or roughly equal to the contribution of cpDNA. Interestingly, the value of morphological data is especially visible within the clade containing *Cyclocarya*, *Pterocarya*, and *Juglans*, three genera that arose during or prior to the Eocene (Manchester, 1987). Although the role of morphological data is clearly evident, convergence in characters related to dispersal biology (i.e., animal dispersal in *Juglans* and *Carya*) appears to be one source of character conflict in the analysis of morphology (see Figs. 8A, 9). This is especially borne out in the lack of morphological/chemical support for Manning's hypothesis (1978) that *Cyclocarya* + *Pterocarya* are closely related to *Juglans*, a robust result of the phylogenetic analysis of two independent molecular data sets (Fig. 8). The placement of *Cyclocarya* is noteworthy given that fossil representatives are among the oldest records for Juglandaceae (see Manchester, 1987; Fig. 1). Extinct juglandaceous fruits such as *Polyptera* and *Cruciptera* appear to share with *Cyclocarya* the condition of a disk-like wing oriented perpendicular to the nut axis (Manchester & Dilcher, 1997). The antiquity of the *Cyclocarya* lineage, coupled with its relationship to more recent genera with derived fruit types, suggests that this character may be plesiomorphic within the Juglandoideae subclade. The well-nested cladistic position of *Cyclocarya* is best explained by numerous extinctions of *Cyclocarya*-like sister lineages (e.g., *Cruciptera*, *Polyptera*) that arose after the divergence of the *Platycarya* lineage.

CLASSIFICATION AND TAXONOMIC CIRCUMSCRIPTION

In comparing our results to previous non-cladistic classifications of the family (Table 1), Iljinskaya's treatment (1990, 1993) is the most consistent in the identification of major clades, though some of the earlier systems contributed significantly to our understanding. Leroy's (1955) Oreomunnoideae and Juglandoideae were based principally on the mode of fruit dispersal, with the former subfamily relying on wind, except for *Alfaroa*, and the latter on animals. Manning's synopsis (1978) emphasized the uniqueness of *Platycarya* by recognizing the monotypic subfamily Platycaryoideae. Schaarschmidt (1985), on the other hand, placed *Platycarya* and *Carya* in the Platycaryeae, and this tribe plus the Engelhardieae in the subfamily Platycaryoideae (Table 1). Following the results of Wing and Hickey (1984), Manchester (1987) also classified *Platycarya* and Engelhardieae in the same subfamily. With the exception of Iljinskaya's treatment, the other classifications depict variable circumscriptions and arrangements within a two-

subfamily concept while consistently recognizing two groupings: (1) *Engelhardia, Alfaroa,* and *Oreomunnea*; (2) *Juglans, Pterocarya,* and *Cyclocarya.* Our results corroborate these groupings, and in addition support *Cyclocarya* as sister to *Pterocarya,* both genera in turn forming the sister group to *Juglans* (Figs. 8D and 9). The position of *Carya* has been somewhat variable in previous classifications. Our results are congruent with those schemes implying a close relationship between *Carya* and the group composed of *Juglans, Pterocarya,* and *Cyclocarya* (Manchester, 1987; Iljinskaya, 1990, 1993).

On the basis of the cladistic analysis presented here, our revised classification (Table 1) recognizes two subfamilies within Juglandaceae, Engelhardioideae and Juglandoideae, and two tribes within the latter, Platycaryeae and Juglandeae. This classification is most similar to that of Iljinskaya (1990, 1993), but differs in treating *Platycarya* as a monotypic tribe within an expanded Juglandoideae on the basis of the numerous synapomorphies shared with the Juglandeae. Under this interpretation, the uniqueness of *Platycarya* as measured in autapomorphies is de-emphasized relative to the robust support for two major lineages within the family. Another notable difference is our decision to recognize only two tribes within subfamily Juglandoideae by expanding Juglandeae to include *Carya* as well. This arrangement is novel and consistent with the morphological distinction between *Platycarya* and its sister clade.

Engelhardioideae Iljinskaya, Bot. Zhurn. (Moscow & Leningrad) 75: 793. 1990. TYPE: *Engelhardia* Lesch. ex Blume.

As noted previously, this clade has had a long and varied history at the tribal level. Iljinskaya (1990: 793) elevated it to the rank of subfamily based on "the makeup of the xylem, inflorescence, bracteoles, and the pollen grains." Autapomorphies identified in Table 3 and Figure 9 lend support to this new subfamily:

Wood with scalariform and simple perforation plates; terminal buds naked; staminate bracts 3-lobed; pistillate bracts 3-lobed; bracteoles 0–2, posterior, fused only on lower half of ovary; nutshell of fibers.

The Engelhardioideae (with a "t," Engelhardtioideae), along with the Platycaryoideae and Juglandoideae, were subsequently accepted by Takhtajan (1997). Recently, however, the *Engelhardia* clade was elevated to family status (Engelhardtiaceae) by Reveal and Doweld (1999), but without discussion. The uncertainty about relationships of this taxon has carried over as well to the genera. *Engelhardia,* for example, was first described from Java in 1825, and *Oreomunnea* was proposed in 1856 based on a Costa Rican collection. Since both of these taxa have trilobed-wing fruits that look superficially similar, many researchers considered *Oreomunnea* as a New World section of *Engelhardia* (e.g., Manning, 1959; Molina, 1968; Rzedowski & Palacios-Chávez, 1977). However, a reassessment of the vegetative and reproductive features led Stone (1972) to resurrect *Oreomunnea,* and subsequently Manchester (1987) showed that this genus has a fossil lineage distinct from *Engelhardia* that extends back into the Oligocene. The generic rank of *Alfaroa* has also been questioned and discussed by Manning (1949), but in the final analysis he concluded that both taxa deserve generic status (Manning, 1978). Certainly the accumulated evidence from the fossil record, morphology, anatomy, chemistry, and the DNA sequence data presented here is in accord on this point. The hypothesis that *Alfaroa* is an animal-dispersed offshoot of the wind-dispersed *Oreomunnea* lineage is fully supported (Stone, 1973; see Figs. 1, 8, 9).

The status of the Old World *Engelhardia* has also been called into question recently by Iljinskaya (1990). In this paper she noted that *E. roxburghiana* sect. *Psilocarpeae* "combines features of *Engelhardia* [sect. *Engelhardia*] with those of *Alfaroa* and partly of *Oreomunnea,* with the predominantly common features being those of *Alfaroa*" (1990: 795). Common characteristics of *E. roxburghiana* and *Alfaroa* include the evergreen habit, presence of androgynous panicles, staminate flowers with rounded receptacles, pollen grains with thick exines, pistillate flowers with absent or rudimentary bracts, and rounded carinal stigma lacking stylar arms. Based on these differences she proposed the taxonomic combination of *Alfaroa roxburghiana* (Wallich) Iljinskaya. Subsequently Iljinskaya (1993) revisited this new combination and stressed its intermediate characteristics between *Engelhardia* and *Alfaroa,* in particular the venation pattern of the trilobed wing and the pollen grains, and concluded that a new monotypic genus was in order, *Alfaropsis roxburghiana* (Wallich) Iljinskaya. The intermediacy of this taxon had been previously recognized (Manning, 1966; Stone, 1972; and see also the bract clearings in Dilcher et al., 1976), but independent evidence to evaluate its status was not available until a sample of *Engelhardia* sect. *Engelhardia* (*E. spicata*) was made available for molecular analyses in this study. While the data are still too limited to draw sweeping conclusions, *E. roxburghiana* shares more in common with *Oreo-*

munnea and *Alfaroa* than with *E. spicata* (Figs. 8D, 9), as Iljinskaya's work would suggest. Clearly, however, a broader sampling of *Engelhardia* is in order before a formal decision can be made about the merits of *Alfaropsis* (Table 1).

Juglandoideae Manning, Ann. Missouri Bot. Gard. 65: 1070. 1978. TYPE: *Juglans* L.

This clade was first recognized as the Drupoideae by Koidzumi (1937) based on the fruits of *Alfaroa, Juglans,* and *Carya.* The name has been rejected as invalid, however, because it was not based on the type genus *Juglans.* Leroy (1955) coined the Juglandoideae epithet, but failed to provide a Latin description. Melchior (1964) also used this subfamily name and acknowledged Koidzumi, but he omitted the full citation of Koidzumi's publication as required at the time by the *International Code of Botanical Nomenclature* (R. L. Wilbur, pers. comm.). Manning's use of the subfamily Juglandoideae (1978) constitutes the first valid use of the name (R. L. Wilbur, pers. comm.). It contrasts with the Engelhardioideae in the following ways:

Wood with strictly simple perforation plates; terminal bud generally with scales; staminate bracts 1-lobed; pistillate bracts unlobed; bracteoles 2, lateral, fused at the middle of ovary or above; nutshell of sclereids.

This clade has two components that we are treating at the tribal level. The Platycaryeae are monotypic with the genus *Platycarya*; the Juglandeae have two subtribes, the Juglandinae subtr. nov. and the Caryinae subtr. nov., the former with three genera (viz., *Juglans, Pterocarya,* and *Cyclocarya*), and the latter with one or two (viz., *Carya* and possibly *Annamocarya*) (Table 1). The Platycaryeae and Juglandeae were both validly published by Nakai (1933). They can be distinguished as follows:

Platycaryeae Nakai, Fl. Sylvatica Koreana 20: 76. 1933. TYPE: *Platycarya* Siebold & Zuccarini.

Staminate bracteoles absent; pollen small (15–16 μm), triangular in polar view, a pair of pseudocolpi on each polar hemisphere; pistillate spike condensed, cone-like; bracts persistent on infructescences, free of fruit.

Juglandeae Nakai, Fl. Sylvatica Koreana 20: 83. 1933. TYPE: *Juglans* L.

Staminate bracteoles generally present; pollen medium to large (28–66 μm), triangular, to circular, to polygonal in polar view, pseudocolpi absent; pistillate spike elongate; bracts attached to and dispersed with the fruit.

Two new subtribes of the Juglandeae are described here for the first time.

Juglandinae Stone & Manos, subtr. nov. TYPE: *Juglans* L., Sp. Pl. 2: 997. 1753.

Medulla locellata; pollenis grana 4(vel+)-pora; bracteolae pistillatae 2, ad medium ovarii connatae; positio carpellorum media (sensu Manningii); stigmata carinalia; papillae stigmaticae in pagina interiori styli elongati bifurcati.

Pith chambered; pollen grains 4 (or more)-pored; pistillate bracteoles 2, fused at the middle of the ovary; position of the carpels median (sensu Manning); stigmas carinal; stigmatic papillae on the inner surface of the elongate, bifurcate style.

Caryinae Stone & Manos, subtr. nov. TYPE: *Carya* Nutt., Gen. N. Am. Pl. 2: 220. 1818, nom. cons.

Medulla solida; pollenis grana 3-pora; bracteolae pistillatae 2 (vel 3–5), ad apicem ovarii connatae; positio carpellorum transversa (sensu Manningii); stigmata commissuralia; papillae stigmaticae paginam stigmatis brevis, rotundati tegentes, stylo obsoleto.

Pith solid; pollen grains 3-pored; pistillate bracteoles 2 (or 3–5), fused to the top of the ovary; position of the carpels transverse (sensu Manning); stigmas commissural; stigmatic papillae covering the surface of the short, rounded stigma, the style obsolete.

The genus *Platycarya* has a number of distinctive features that clearly set it apart from all other genera in the family and a number of "advanced" traits that link it with the walnuts and hickories. The pollen, for example, is unique in the angiosperms with a pair of arcuate pseudocolpi (crease-like thinnings of the exine: Manchester, 1987) on each polar hemisphere (Whitehead, 1965; Stone & Broome, 1975). The wood, fruit, and chromosome number provide additional features as well. The wood has spiral thickenings in the secondary walls of both the small vessel elements and the vascular tracheids (Heimsch & Wetmore, 1939; Manchester, 1987). The infructescence looks like a small cone with persistent sharp-pointed bracts subtending small wind-borne seeds (Wing & Hickey, 1984; Endress, 1986; Manchester, 1987). The chromosome number of N = 15 (Fig. 7) is also unique in a family with a base number of $x = 16$ (see Karyology). Traits shared with most of the Juglandeae include short, large-diameter vessel elements with simple perforation plates (Heimsch & Wetmore, 1939), the deciduous habit, terminal bud scales, odd-pinnate leaves, leaflets with serrate margins,

druse crystals in the leaf palisade, and transverse orientation of the carpel.

The five Juglandeae taxa have two of the best known genera in the family (viz., *Juglans* and *Carya*) and two of the least known. New information on the least known lends credence to the recognition of *Cyclocarya* and casts doubt on the distinctness of *Annamocarya*. *Cyclocarya* was proposed by Iljinskaya (1953) as a segregate from *Pterocarya* (*P. paliurus* Batalin) based on a different mode of fruit wing formation (bract fuses with the 2 bracteoles to produce the circular wing versus bract free and not incorporated into the 2-winged fruit), plus a number of other distinguishing features such as staminate catkins (cluster of 3 or more versus solitary), leaflet venation (some lateral veins pass directly into the teeth versus all veins connected with each other by loops), and pollen (3- to 4-porate versus 5- to 7-porate). This original treatise was in Russian and essentially inaccessible to many western botanists until the National Science Foundation facilitated an English version through the Israeli Program for Scientific Translation. Manning (1975) was the first to take advantage of the translation and to provide an analysis of Iljinskaya's newly proposed genus. After a detailed examination of the character differences Manning concluded that most are variable or of minor importance. He did nevertheless elevate the taxon to subgeneric rank as *Cyclocarya* (Iljinskaya) Manning. Iljinskaya addressed the issue again in 1990 and reiterated that the basic differences between *Cyclocarya* and *Pterocarya* are found in the mode of fruit wing development. At the same time, however, she determined that KOH clearings of fresh young (circular) wings of *Cyclocarya* fruits revealed that all of the veins are uniform and radially distributed, and hence provide no evidence as to the role the bract and two bracteoles play in wing development (but see below).

The open question about the exact nature of the relationship of the bract and bracteoles in *Cyclocarya* has been due principally to the unavailability of flowering material. Now for the first time we are able to provide additional insight into floral morphology of this taxon based on developmental sequences secured from a tree growing in the J. C. Ralston Arboretum (Table 2). While the material is revealing and clearly shows the relationship between the "tooth-shaped" inferior bract and the superior bracteoles united in a circular disk (Fig. 6A), Iljinskaya's description of the *Cyclocarya* flower is basically correct: "the lower part of the ovary is concrescent with the fused bases of the bract and bracteoles, and from the middle of the ovary a narrow border diverges consisting of the fused upper parts of bract and bracteoles; this border is wider in the place where the bract should have been, and its edge has the shape of a flat tooth [see Fig. 6D]. As the fruit develops, the border of the ovary increases very considerably and forms a coriaceous disk around the fruit . . ." (see Manning, 1975: 158). She then goes on to say that the edges of the disk become uneven as the fruit matures and the tooth becomes indistinguishable. Taking exception, Manning (1975) noted that it is not uncommon to find herbarium specimens with mature fruits that retain the tooth (i.e., scale-like apex of the bract). While this observation seems perfectly reasonable, Iljinskaya (1990: 792) was adamant that "the tooth on the mature fruits discovered by Manning can never be considered a constant element of the fruit of *Cyclocarya*." Our observations in this case support Manning. Mature fruits displaying a tiny (ca. 1 mm) tooth on the abaxial perimeter of the disk opposite the point of fruit attachment (Fig. 6C), though not common, were routinely observed in our sampling. The absence of the bract in many mature fruits could be due, as Manning suggested, to breakage during growth or processing of the specimen, but our observations suggest that variability in the fusion of the bract and the bracteoles is also responsible. What one sees when the flower is viewed from the side is that the bract is clearly inferior (below) to the rim of the bracteole disk in some (Fig. 6A), and approximately on the same level in others, thus forming a small beak or tooth. We presume that the latter type is more likely to retain the tooth as a visible entity in the mature fruit (Fig. 6C). This brings us back to a point noted previously in which Iljinskaya (1990) stated that the veins of the circular wing of *Cyclocarya* are radially distributed and uniform, thus ruling out the possibility of the bract participating with the bracteoles in the formation of the wing. Not only do we find the bract present in many well-preserved mature fruits, but the bracteole margins have been shown to have a loose arrangement of terminating veins, whereas the tooth-like bract is clearly supplied with two parallel vascular strands (Fig. 6D).

Fresh flowers of *Cyclocarya* also permitted assessment of the orientation of the carpel and stigma. In a sample of 12 flowers the majority had carpels with a median orientation (see discussion of character 33), transverse carpels were found in two of them, and orientations between median and transverse were also observed occasionally. The bifurcate stigma is oriented opposite the carpel sutures of the primary partition, so that stigma is always commissural irrespective of whether the carpels are median or transverse. Technical mor-

phological details aside, there is still the question of the validity of *Cyclocarya* as a genus. There is strong independent evidence from ITS and weak evidence from cpDNA (Fig. 8) that *Cyclocarya* is a distinct clade. The most convincing evidence, however, comes from our chromosome count of N = 28 (Fig. 7A), which is unique for the family (see Karyology).

Annamocarya is perhaps the least known and most questionable member of the family. It was described in 1941 by Chevalier (September–October), and in November of the same year Kuang described *Rhamphocarya integrifoliolata*. Both were treated by Manning and Hjelmqvist (1951) as synonyms of *Carya sinensis* Dode (1912) and placed in the new section *Rhamphocarya*. The uncertain status that has continued to plague this taxon was reflected initially in Chevalier's decision to describe a fruit collection as *Annamocarya indochinensis* and another collection with leaves and nuts that lacked the husk as *Juglans indochinensis*. According to Manning and Hjelmqvist (1951: 319), "This author apparently felt dubious, whether the species described should be referred to this new genus or to *Juglans* . . . ." He clearly recognized that only one taxon was represented here, but provided no resolution to the quandary. Adding to the confusion is the question why Chevalier did not relate the specimens of his new genus to those of Dode's *Carya sinensis*, since the fruit illustrations found in both papers are remarkably similar. Chevalier certainly knew of Dode's new species (1941: 506), but he seemed to defer to Lecomte (1921) as the expert in the matter. It could be that Chevalier did not actually see Dode's paper, for it is not cited in the bibliography. Lecomte (1921: 437), on the other hand, examined the nut studied by Dode and was able to confirm that it is a member of the Juglandaceae, "but having only seen the nut, we cannot pass judgment on the genus."

While the entire-margined leaflets of *Annamocarya* superficially resemble *Juglans regia*, the question of taxonomic rank really depends on its relationship to other sections of *Carya* (e.g., *Apocarya, Carya,* and *Sinocarya*; Lu et al., 1999). *Annamocarya* shares the Caryinae features noted above with *Carya,* but differs in a number of significant ways: leaflets entire (versus serrate in *Carya*); husk 4- to 9-valved (versus 4, rarely 3 to 6: Grauke et al., 1991); dorsal vascular bundles in shell wall (versus primary partition: Leroy, 1955); basal plexus of nut prominent (versus poorly developed); stigmatic disk absent (versus present); catkins from axils of foliage leaves (versus bud scales); catkin clusters 5 to 8 (versus 3, or very rarely 4 to 7); staminate bract with 3 or more vascular strands (versus 1); and stamens 5 to 15 (versus 4, or occasionally 3 to 8). Features that serve to separate *Annamocarya* from *Carya* are of greater significance than those used to recognize sections of *Carya,* where the most distinctive character is the terminal bud, which is pseudovalvate in section *Apocarya* with 4 to 6+ scales, imbricate in section *Carya* with 6 to 12 scales, and naked in section *Sinocarya* (Kuang & Lu, 1979). Recent studies have revealed, however, that the bud scales of all Juglandaceae are organized on a phyllotactic spiral, and those buds that appear valvate (i.e., decussate) do so only because the scales are relatively narrow and few in number (Stone, unpublished). *Annamocarya* buds are most similar in morphology to those of section *Apocarya*; most other features used to recognize the sections intergrade. There is a general tendency for more leaflets, sometimes falcate, in section *Apocarya* (7 to 17), whereas section *Carya* has 6 to 12 leaflets, section *Sinocarya* 7 to 9, and the genus *Annamocarya* 7 to 11. Section *Apocarya* also has a propensity for developing staminate catkins from lateral buds on the preceding season's shoots, and lacunae are characteristic of the shell wall (Manning, 1978; Stone, 1997). The other sections, however, bear staminate catkins in a narrow band at the base of leafy shoots (scattered along the base of the shoot in *Annamocarya*), and the shell wall is thick and solid. With a relatively weak set of features to differentiate the three sections of *Carya* and a significant number of developmentally complex characters distinguishing *Carya* from *Annamocarya,* Lu et al. (1999) concluded that both taxa merit recognition at the generic level. This decision, however, antedated the current work that provides an independent assessment of relationships based on molecular data and integrates this information with morphology and chemistry in the cladistic analyses. The picture now is far less supportive. There are certainly plenty of differences between *Annamocarya* and *Carya,* as noted above, but to date no autapomorphies have been identified. Then, too, the scarcity of material of *Annamocarya* has limited the depth of investigations. As of now, for example, no chromosome number has been reported for this monotypic taxon and it is not at all clear if *Annamocarya* lacks the stigmatic disk present in *Carya*. The molecular data and combined data set (Figs. 8D, 9) suggest that *Annamocarya* is a close ally of the sympatric Southeast Asian species of *Carya cathayensis* and *C. tonkinensis* and distinct from North American *Carya*. While it seems likely now that *Annamocarya* is best treated as a section of *Carya,* as proposed by Manning and

Hjelmqvist (1951), we defer action on this matter until a wider sampling of Southeast Asian species is available for study.

KARYOLOGY

The core "Higher" Hamamelididae (Fig. 2) have a base chromosome number ranging from $x = 8$ (Myricaceae), $x = 9$ and 11 (Casuarinaceae), $x = 13$ (Ticodendraceae), $x = 14$ (Betulaceae), to $x = 16$ (Oginuma, 1999). The Rhoipteleaceae and Juglandaceae sensu Cronquist (1981) both share the base number of 16 and some similarities in their chromosome morphology. A study of the karyotype of *Rhoiptelea* revealed that the first and second longest chromosomes have secondary constrictions at the median to submedian position (Oginuma et al., 1995), as do the Juglandaceae represented by studies of *Carya, Juglans, Platycarya,* and *Pterocarya* (Oginuma & Tobe, 1992). The Myricaceae, with $x = 8$, are the only other member of the core group that has obvious similarities, with the longest chromosome also bearing a secondary constriction.

The chromosome picture is a bit more complicated than noted above, however, due to the fact most families have undergone polyploidy followed by chromosome loss. The Juglandaceae are no exception. The haploid number of 16 holds for Engelhardioideae (Fig. 9), but the Juglandoideae have diploids, polyploids, and aneuploids (Stone, 1993). *Platycarya,* for example, is as distinctive in its chromosome number as it is in many other traits, though the exact count of this monotypic taxon is questionable. Morawetz and Samuel (1989) first reported $2n = 28$. More recently Oginuma and Tobe (1992) found $2n = 30$ and illustrated the karyotype, whereas Wu (1995) reported $2n = 22$ and Hsu et al. (1994) counted $2n = 24$. We had the opportunity to examine meiosis in pollen mother cells of a local ornamental tree over a period of several weeks during the months of May and June. Acetocarmine squashes yielded excellent slides and photographs of diakinesis and metaphase I. To our consternation, however, the counts were variable with both N = 15 (Fig. 7B) and N = 14 (Fig. 7C). After checking some 18 clearly countable cells, two-thirds were determined to have N = 15. The basis for this difference is not known, though it is possible that two of the bivalents may occasionally clump (see legend to Fig. 7C). The logical follow-up will be to check the mitotic chromosome number.

The genus *Carya* has both N = 16 and N = 32 (Stone, 1993). The pecan hickories, section *Apocarya,* are diploid, whereas the true hickories, section *Carya,* have both N = 16 (*C. myristiciformis, C. laciniosa,* and *C. ovata*) and tetraploid N = 32 (*C. floridana, C. glabra, C. pallida, C. texana,* and *C. tomentosa).* The one species of section *Sinocarya* from Southeast Asia for which a count has been published, *C. cathayensis* (Chen, 1993), is N = 16. An unconfirmed count of N = 24 for *C. hunanensis* (Grauke, pers. comm.) gives this species triploid status. The closely related *Annamocarya* (= *C. sinensis,* sect. *Rhamphocarya*) has not been counted to our knowledge.

Our biggest surprise of all came when we examined the Juglandinae alliance of *Juglans, Pterocarya,* and *Cyclocarya.* The many species of *Juglans* and *Pterocarya* that have been counted are diploid with N = 16, and so it was quite unexpected when the monotypic *Cyclocarya* displayed N = 28 (Fig. 7A). This is the first report of a chromosome count for the genus and, fortunately, it provides a convincing autapomorphy confirming the distinctness of *Cyclocarya* from its sister genus *Pterocarya* (Fig. 9).

ECOLOGICAL ADAPTATIONS

Several distinctive characteristics or syndromes common to various sets of genera are clearly due to convergences of morphological or chemical features that are best explained in terms of ecological adaptations associated with climate, wind versus animal dispersal, or open versus closed habitats. The leaflet margin is a conspicuous vegetative trait that has been subject to strong environmental pressures, whereas seed-energy reserves, dispersal, and germination are reproductive features that also display ecological adaptations leading to multiple evolutionary origins. Leaflet margins follow the broad-scale vegetational pattern noted by Bailey and Sinnott (1916) and others, namely that areas of high mean annual temperatures and precipitation (viz., the tropics) have a preponderance of plants with entire leaf margins. Conversely, leaves of cool climates have a higher percentage of small leaves with toothed margins (Wolfe, 1978, 1995). An examination of character 7, for example, shows that the Engelhardioideae (Fig. 9) are characterized by leaflets with entire margins. This is true of the tropical American species of *Alfaroa* and *Oreomunnea* and mainly true of the Southeastern Asian species of *Engelhardia.* Qualifications are in order, however, that may reflect the retention of ancestral traits or fine-scale ecological adaptations. The leaflets on mature trees of *Alfaroa* and *Oreomunnea* are entire, though sucker shoots may be serrate in some species. Similarly, the seedlings and young saplings of

some species (e.g., *A. costaricensis*, *A. williamsii*, and *O. mexicana*) have relatively thin, serrate leaflets while others (e.g., *A. guanacastensis*, *A. manningii*, and *O. pterocarpa*) are thicker and entire, as would probably be predicted by Givnish (1979) based on leaf thickness alone. The monotypic *Engelhardia roxburghiana* (sect. *Psilocarpeae*) is a tree with entire leaflets that occupies a broad range of habitats. It is a primary forest tree in the hilly country of Malaysia (Jacobs, 1960), and in China it is part of the mixed broad-leaved or evergreen forests on loam, or situated on steep dry slopes with sandy soil (Lu et al., 1999). *Engelhardia serrata* (sect. *Engelhardia*) with crenate or serrate leaflets, on the other hand, occurs in Malaysia in primary upland forest on sandy or clayey soil (Jacobs, 1960). The serrate leaflet is invariant in the temperate-zone taxa of *Juglans*, *Pterocarya*, *Cyclocarya*, *Carya*, and *Platycarya*, but *Juglans regia* (monotypic in sect. *Juglans*) and the monotypic *Annamocarya* both have entire leaflets (Fig. 9). Certainly *Annamocarya* is tropical, but the origin of the widespread Persian Walnut is debatable. A comment is in order here about the extension of the *Rhysocaryon* section of *Juglans* from North America south through Mesoamerica (skipping Costa Rica and Panama), and down into South America as far as the northern reaches of Argentina (see Manchester, 1987). This black walnut group of species with serrate leaflets ranges across the tropical zone, but it does so by occupying montane regions that have distinctly temperate climates.

Seed-energy reserves are associated with the enhancement of seed germination and seedling survival. As Levin (1974: 193) emphasized, "the larger the seed the greater the energy reserve and the greater the probability of seedling establishment during low illumination or high competition . . . ." Independent of large seeds, however, natural selection has favored the storage of lipids because the oxidative metabolism of reserves yields over twice as much energy as that of an equivalent weight of carbohydrate or dry protein (Levin, 1974; Slabas et al., 1993). This suggests, therefore, that lipid storage would be advantageous in both small seeds where weight is a factor in wind dispersal and large seeds where animals place special demands on the seeds and seedlings (Stone, 1973, 1989). In this regard the nuts of *Carya*, *Juglans*, and *Corylus* of the Betulaceae are "in a class by themselves as high protein, high fat, and low carbohydrate foods . . ." (Wainio & Forbes, 1941: 634) and are considered to be the quintessential animal-dispersed fruits. The seeds of *Rhoiptelea* and the Juglandaceae have stored energy reserves consisting of five fatty acids: palmitic, stearic, oleic, linoleic, and linolenic. Both palmitic and stearic are saturated fatty acids, whereas oleic, linoleic, and linolenic are unsaturated. In plants in general, oleic and linoleic account for 80% of the fatty acids, palmitic amounts for less than 10%, and all other unsaturated and saturated acids make up the balance (Shorland, 1963; Hilditch & Williams, 1964). There is a functional correlation between cold climates and unsaturated fatty acids and the reciprocal relationship of warm climates and saturated fatty acids that is based on the fact that "seed fats must be fluid at the temperature of the living plant" (Hilditch & Williams, 1964: 206). This means that unsaturated acids such as oleic and linoleic will predominate in the cool temperate zone and higher percentages of palmitic would be expected to occur in tropical or subtropical climates (McNair, 1929). This generalization corresponds rather well to the composition of seed fats of *Rhoiptelea* and the Juglandaceae (Table 4) and correlates with the serrate versus entire leaflet margins. The saturated palmitic fatty acid, for example, is generally high in *Engelhardia* (information is only available for *E. roxburghiana*), *Oreomunnea*, *Alfaroa*, and *Annamocarya*, and these are the same tropical taxa that possess leaflets with entire margins. Low palmitic acid percentages characterize the temperate, serrate-leaflet genera of *Platycarya*, *Carya*, *Cyclocarya*, *Pterocarya*, and *Juglans*, all members of the Juglandoideae (Table 4), as well as *Rhoiptelea*. The only exception to the above statements was encountered in samples of *Engelhardia roxburghiana*, one based on a collection from Taiwan and analyzed by G. A. Adrouny (high palmitic), and the other reported in Jiang and Zhou (1990) for Chinese material (low palmitic). There is no way to know if the difference is due to technique, phenotypic variability, or genetic differences. However, as others found (Stone et al., 1965; Stone et al., 1969; Rogers, 1972) and Hohn and Meinschein (1976: 193) noted, it "does not appear that environmental influences . . . greatly mask the characteristic, genetically-controlled fatty acid profile of a given species." We are dealing here perhaps with results expected of a species capable of ranging from cool temperate to tropical climates.

Modes of seed dispersal are also correlated with the seed-energy reserves, and the evolution of wind-dispersed and animal-dispersed fruits has occurred independently several times. The three unsaturated fatty acids labeled here A (oleic), B (linoleic), and C (linolenic) (Table 4) can be used for the most part to characterize each of the genera based on the percentage of the two most abundant acids. For example, with the exception of *Oreomunnea*, all wind-dispersed fruits have the CB pat-

tern of fatty acid compostion. This includes *Rhoiptelea, Platycarya, Cyclocarya,* and *Pterocarya.* Conversely, all animal-dispersed fruits have the BA or AB pattern, including *Alfaroa, Carya,* and *Juglans. Oreomunnea* is an interesting exception. It has the pleisomorphic trait of fruit wings and wind dispersal and yet is sister to *Alfaroa,* which bears animal-dispersed nuts. It would appear that whatever advantages or constraints the BA pattern confers, ancestors of the *Oreomunnea* lineage (see Manchester, 1987) were preadapted to the evolution of animal-dispersed fruits. *Annamocarya,* a taxon that some consider to be a section of *Carya* [sect. *Rhamphocarya* (Kuang) Manning & Hjelmqvist], is BA (like *Juglans*), whereas *Carya* (including the Chinese sect. *Sinocarya*) has the AB fatty acid pattern.

Wind dispersal is found in the outgroup *Rhoiptelea* (Wu & Kubitzki, 1993), whereas in the Juglandaceae four different patterns account for the independent origin of wings in five genera, and animal dispersal characterizes four genera and three developmental pathways that lead to the production and convergence on wingless nuts (see Fig. 9). All of these fruit types arise from a floral envelope consisting of an adaxial bract, two lateral bracteoles, and four sepals. In the trilobed fruit dispersal unit of *Engelhardia* and *Oreomunnea,* the wings are formed by the tripartite bract (see character 43-1). In *Platycarya,* the small outgrowths producing the 2-winged fruit result from the fusion of the bracteoles and lateral sepals (see 43-4). In *Pterocarya,* the two wings are strictly bracteole in origin (see 43-3), whereas the circular wing of *Cyclocarya* arises from the fusion of the bract and bracteoles (see 43-2). In the animal-dispersed taxa the nut husk has evolved uniquely from different accessory structures: fusion of four sepals in *Alfaroa* (43-5); fusion of the bract, bracteoles, and sepals in *Juglans* (43-6); and fusion of the bract and bracteoles in *Carya* and probably *Annamocarya* (43-7).

The syndromes associated with the modes of dispersal quite naturally carry over to seed germination and seedling establishment, and convergences are the rule (Fig. 9). The relatively light, wind-dispersed seeds that are best suited to open habitats (viz., stream banks, plains, open hillsides, canopy gaps) have limited food reserves and epigeal germination (Conde & Stone, 1970; but see Iljinskaya, 1990). In this context, exposing the cotyledons to light and activating photosynthesis is critical to producing vigorous, competitive seedlings (Stone, 1989). The heavier, animal-dispersed seeds from forest-dwelling trees are capable of establishing vigorous seedlings in closed, shaded habitats (Stebbins, 1974; Foster, 1986). All of these wingless nuts have hypogeal germination, but two genera are exceptions to these general patterns. The trilobed *Oreomunnea* fruit has hypogeal germination, like its animal-dispersed sister genus *Alfaroa,* in contrast to the trilobed *Engelhardia,* which is epigeal. In the case of *Alfaroa,* the hypogeal state is almost certainly due to the evolution of the animal-dispersed fruit. However, the preadaptive nature of *Oreomunnea* seeds with respect to the BA pattern of fatty acids further supports a single origin of hypogeal germination in the ancestor of this New World subclade of Engelhardioideae.

The second exception occurs within Juglandoideae; the circular-winged fruit of *Cyclocarya* is reported to have hypogeal germination (Iljinskaya, 1990), unlike its sister genus *Pterocarya,* which displays epigeous cotyledons (Conde & Stone, 1970). In *Cyclocarya,* hypogeal germination is presumably a functional adaptation that accommodates the large circular wing. In the phylogenetic context provided, parsimony favors a gain of hypogeal germination on the branch above *Platycarya* and a reversal to epigeal germination in *Pterocarya* (Fig. 9, see character 46-1 > 46-0). Although three independent gains of hypogeal germination appears to make greater biological sense in the evolution of *Carya, Cyclocarya,* and *Juglans,* the more parsimonious solution could be the result of extinction of wind-dispersed taxa along the branch leading to *Carya.*

Literature Cited

Allard, M. W. & J. M. Carpenter. 1996. On weighting and congruence. Cladistics 12: 183–198.

Bailey, I. W. & E. W. Sinnott. 1916. The climatic distribution of certain types of angiosperm leaves. Amer. J. Bot. 3: 24–39.

Berquam, D. L. 1975. Floral Morphology and Anatomy of Staminate Juglandaceae. Ph.D. Thesis, University of Minnesota, St. Paul.

Bolick, M. R. 1983. A cladistic and biogeographic analysis of the Juglandaceae. Amer. J. Bot. 70 (Suppl.): 106.

Bos, J. A. A. & W. Punt. 1991. Juglandaceae. Rev. Palaeobot. Palynol. 69: 79–95.

Candolle, C. de. 1862. Mémoire sur la famille des Juglandées. Ann. Sci. Nat. Bot. 18: 5–48, + 6 plates.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y.-L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedrén, B. S. Gaut, R. K. Jansen, K.-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q.-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan & V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.

Chen, R.-Y. (editor). 1993. Chromosome Atlas of Chinese Fruit Trees and Their Close Wild Relatives. Chromosome Atlas of Chinese Principal Economic Plants 1. China International Academic Publishers, Beijing.

Chen, Z.-D., X.-Q. Wang, H.-Y. Sun, Y. Han, Z.-X. Zhang, Y.-P. Zou & A.-M. Lu. 1998. Systematic position of the Rhoipteleaceae: Evidence from nucleotide sequences of *rbc*L gene. Acta Phytotax. Sin. 36: 1–7.

Chevalier, A. 1941. Varabilité et hybridité chez les noyers. Notes sur des *Juglans* peu connus, sur l'*Annamocarya* et un *Carya* d'Indochine. Rev. Bot. Appl. Agric. Trop. 21: 477–509.

Conde, L. F. & D. E. Stone. 1970. Seedling morphology in the Juglandaceae, the cotyledonary node. J. Arnold Arbor. 51: 463–477.

Cronquist, A. 1968. The Evolution and Classification of Flowering Plants. Houghton Mifflin, Boston.

———. 1981. An Integrated System of Classification of Flowering Plants. Houghton Mifflin, Boston.

DeSalle, R. & A. V. Z. Brower. 1997. Process partitions, congruence, and the independence of characters: Inferring relationships among closely related Hawaiian *Drosophila* from multiple gene regions. Syst. Biol. 46: 751–764.

Dilcher, D. L. 1974. Approaches to the identification of angiosperm leaf remains. Bot. Rev. 40: 1–157.

——— & S. R. Manchester. 1986. Investigations of angiosperms from the Eocene of North America: Leaves of the Engelhardieae (Juglandaceae). Bot. Gaz. 147: 189–199.

———, F. W. Potter, Jr. & W. L. Crepet. 1976. Investigations of angiosperms from the Eocene of North America: Juglandaceous winged fruits. Amer. J. Bot. 63: 532–544.

Dode, L. A. 1912. Deux genres nouveaux pour la Chine. Bull. Soc. Dendrol. France No. 23: 58–61.

Eames, A. 1961. Morphology of the Angiosperms. McGraw-Hill, New York.

Endress, P. K. 1986. An entomophily syndrome in Juglandaceae: *Platycarya strobilacea*. Veröff. Geobot. Inst. ETH Stiftung Rübel Zürich 87: 100–111.

Engler, A. 1889. Juglandaceae. Pp. 19–25 *in* A. Engler & K. Prantl (editors), Die natürliche Pflanzenfamilien, Teil III, 1.

Erdtman, G. 1966. Pollen Morphology and Plant Taxonomy. Hafner, New York.

Faegri, K. & J. Iversen. 1964. Textbook of Pollen Analysis, 2nd ed. Munksgaard, Copenhagen.

Farris, J. S., M. Kallersjö, A. G. Kluge & C. Bult. 1994. Testing significance of incongruence. Cladistics 10: 315–319.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

Fjellstrom, R. G. & D. E. Parfitt. 1994. Walnut (*Juglans* spp.) genetic diversity determined by restriction fragment length polymorphisms. Genome 37: 690–700.

——— & ———. 1995. Phylogenetic analysis and evolution of the genus *Juglans* (Juglandaceae) as determined from nuclear genome RFLPs. Pl. Syst. Evol. 197: 19–32.

Foster, S. A. 1986. On the adaptive value of large seeds for tropical moist forest trees, a review and synthesis. Bot. Rev. 52: 260–299.

Givnish, T. J. 1979. On the adaptive significance of leaf form. Pp. 375–407 *in* O. T. Solbrig, S. Jain & P. H. Raven (editors), Topics in Plant Population Biology. Columbia Univ. Press, New York.

Grauke, L. J., B. Wood & J. Payne. 1991. Genetic resources of *Carya* in Vietnam and China. Ann. Rep. North. Nut Growers' Assoc. 82: 80–87.

Gunter, L. E., G. Kochert & D. E. Giannasi. 1994. Phylogenetic relationship of the Juglandaceae. Pl. Syst. Evol. 192: 11–29.

Handel-Mazzetti, H. 1932. Rhoipteleaceae, eine neue Familie der Monochlamydeen. Feddes Repert. 30: 75–80, + 2 plates.

Hardin, J. W. 1981. Atlas of foliar surface features in woody plants, II. *Broussonetia*, *Morus*, and *Maclura* of North America. Bull. Torrey Bot. Club 108: 338–346.

——— & D. E. Stone. 1984. Atlas of foliar surface features in woody plants, VI. *Carya* (Juglandaceae) of North America. Brittonia 36: 140–153.

Heimsch, C. & R. H. Wetmore. 1939. The significance of wood anatomy in taxonomy of the Juglandaceae. Amer. J. Bot. 26: 651–660.

Hilditch, T. P. & P. N. Williams. 1964. The Chemical Constitution of Natural Fats, 4th ed. Wiley, New York.

Hodges, S. A. & M. L. Arnold. 1994. Columbines: A geographically widespread species flock. Proc. Natl. Acad. Sci. U.S.A. 91: 5129–5132.

Hohn, M. E. & W. G. Meinschein. 1976. Seed oil fatty acids: Evolutionary significance in the Nyssaceae and Cornaceae. Biochem. Syst. Ecol. 4: 193–199.

Hsu, P.-S., R.-F. Weng & S. Kurita. 1994. New chromosome counts of some dicots in the Sino-Japanese region and their systematics and evolutionary significance. Acta Phytotax. Sin. 411–418.

Hufford, L. 1992. Rosidae and their relationship to other nonmagnoliid dicotyledons: A phylogenetic analysis using morphological and chemical data. Ann. Missouri Bot. Gard. 79: 218–248.

——— & P. R. Crane. 1989. A preliminary phylogenetic analysis of the 'lower' Hamamelidae. Pp. 175–192 *in* P. R. Crane & S. Blackmore (editors), Evolution, Systematics, and Fossil History of the Hamamelidae, Vol. 1, Introduction and 'Lower' Hamamelidae. Syst. Assoc. Special Vol. 40A. Clarendon Press, Oxford.

Iljinskaya, I. A. 1953. Monograph of the genus *Pterocarya* Kunth. Trudy Bot. Inst. Akad. Nauk SSSR, Ser. 1, Fl. Sist. Vyss. Rast. 10: 7–123. [In Russian, translated, U.S. Dept. Commerce, IPST Catalog 5622.]

———. 1990. On the taxonomy and phylogeny of the family Juglandaceae. Bot. Zhurn. (Moscow & Leningrad) 75: 792–803. [In Russian with English summary, translated by B. M. Johnson, Duke Univ.]

———. 1993. *Alfaropsis*, a new genus of the Juglandaceae. Bot. Zhurn. (Moscow & Leningrad) 78: 79–83. [In Russian with English summary, translated by B. M. Johnson, Duke Univ.]

Jacobs, M. 1960. Juglandaceae. Pp. 143–154 *in* C. G. G. J. van Steenis (editor), Flora Malesiana, I. 6. Noordhoff, Leyden.

Jiang, Z.-H. & R.-H. Zhou. 1990. An analysis of fatty acids in the seeds of Rhoipteleaceae and the implication for its systematic position. J. Chin. Folk Mater. Med. 1990: 33–35. [In Chinese, translated by Y. Tao, Duke Univ.]

Kluge, A. G. 1989. A concern for evidence and the phylogenetic hypothesis of relationship among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38: 7–25.

——— & A. J. Wolfe. 1993. Cladistics: What's in a word. Cladistics 9: 183–199.

Koidzumi, G. 1937. On the classification of the Juglandaceae. Acta Phytotax. Geobot. 6: 1–7. [In Japanese and Latin.]

Kuang, K.-Z. 1941. Genus novum Juglandacearum ex Austro-Orientali Yunnan. Iconogr. Fl. Sin. 1: 1–3, + 1 plate.

——— & A.-M. Lu. 1979. Juglandaceae. Pp. 6–44 *in* K.-Z. Kuang & P.-C. Li (editors), Flora Reipublicae Popularis Sinicae, Vol. 21. [In Chinese, translated by R. B. Essig & U.-P. Hu Essig.]

Lecomte, M. H. 1921. Une Juglandacée du genre *Carya* en Indochine. Bull. Mus. Natl. Hist. Nat. 27: 437–440.

Leroy, J. F. 1953. La structure du bois d'*Annamocarya*. Notes sur le bois des noyers et autres Juglandaceés. Rev. Int. Bot. Appl. Agric. Trop. 33: 216–220.

———. 1955. Étude sur les Juglandaceae. Mém. Mus. Natl. Hist. Nat., Sér. B., Bot. 6: 1–246.

Levin, D. A. 1974. The oil content of seeds: An ecological perspective. Amer. Naturalist 108: 193–206.

Lu, A.-M. 1982. On the geographical distribution of the Juglandaceae. Acta Phytotax. Sin. 20: 257–274. [In Chinese with English summary.]

———, D. E. Stone & L. J. Grauke. 1999. Juglandaceae. Pp. 277–285 *in* Z.-y. Wu & P. H. Raven (editors), Flora of China, Vol. 4. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Manchester, S. R. 1987. The Fossil History of the Juglandaceae. Monogr. Syst. Bot. Missouri Bot. Gard. 21: 1–137.

———. 1999. Biogeographical relationships of North American Tertiary floras. Ann. Missouri Bot. Gard. 86: 472–522.

——— & D. L. Dilcher. 1997. Reproductive and vegetative morphology of *Polyptera* (Juglandaceae) from the Paleocene of Wyoming and Montana. Amer. J. Bot. 84: 649–663.

Manning, W. E. 1926. The Morphology and Anatomy of the Flowers of the Juglandaceae. Ph.D. Thesis, Cornell University, Ithaca, New York.

———. 1938. The morphology of the flowers of the Juglandaceae, I. The inflorescence. Amer. J. Bot. 25: 407–419.

———. 1940. The morphology of the flowers of the Juglandaceae, II. The pistillate flowers and fruit. Amer. J. Bot. 27: 839–852.

———. 1948. The morphology of the flowers of the Juglandaceae, III. The staminate flowers. Amer. J. Bot. 35: 606–621.

———. 1949. The genus *Alfaroa*. Bull. Torrey Bot. Club 76: 196–209.

———. 1959. *Alfaroa* and *Engelhardtia* in the New World. Bull. Torrey Bot. Club 86: 190–198.

———. 1966. New combinations and notes on *Engelhardia* (Juglandaceae) of the Old World. Bull. Torrey Bot. Club 93: 34–52.

———. 1975. An analysis of the genus *Cyclocarya* Iljinskaya (Juglandaceae). Bull. Torrey Bot. Club 102: 157–166.

———. 1978. The classification within the Juglandaceae. Ann. Missouri Bot. Gard. 65: 1058–1087.

——— & H. Hjelmqvist. 1951. *Annamocarya*, *Rhamphocarya*, and *Carya sinensis*. Bot. Not. 4: 319–330.

Manos, P. S. & K. P. Steele. 1997. Phylogenetic analyses of 'higher' Hamamelididae based on plastid sequence data. Amer. J. Bot. 84: 1407–1419.

———, J. J. Doyle & K. C. Nixon. 1999. Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). Molec. Phylogenet. Evol. 12: 333–349.

———, K. C. Nixon & J. J. Doyle. 1993. Cladistic analysis of chloroplast DNA restriction site from the inverted repeat region of 'higher' Hamamelididae. Syst. Bot. 18: 551–562.

McNair, J. B. 1929. The taxonomic and climatic distribution of oils, fats, and waxes in plants. Amer. J. Bot. 16: 832–841.

Melchior, H. 1964. Juglandaceae. Pp. 41–44 *in* A. Engler's Syllabus der Pflanzenfamilien. Ed. 12, Vol. 2. Gebrüder Borntraeger, Berlin.

Metcalfe, C. R. & L. Chalk. 1950. Anatomy of the Dicotyledons, Vol. 2. Clarendon Press, Oxford.

Mickevich, M. F. & J. S. Farris. 1981. The implication of congruence in *Menidia*. Syst. Zool. 30: 351–370.

Miller, R. B. 1976. Wood anatomy and identification of species of *Juglans*. Bot. Gaz. 137: 368–377.

Molina, A. 1968. Two Nicaraguan Juglandaceae. Fieldiana, Bot. 31: 357–359.

Morawetz, W. & M. R. A. Samuel. 1989. Karyological patterns in the Hamamelidae. Pp. 129–154 *in* P. R. Crane & S. Blackmore (editors), Evolution, Systematics, and Fossil History of the Hamamelidae, Vol. 1. 'Lower' Hamamelidae. Syst. Assoc. Special Vol. 40A. Clarendon Press, Oxford.

Nakai, T. 1933. Juglandaceae Lindley. Pp. 66–88, + 3 plates *in* Flora Sylvatica Koreana, Vol. 20. Forest Experiment Station, Government general Chosen, Keijyo, Korea.

Nicoloff, M. T. 1904–1905. Sur le type floral et le développement du fruit des Juglandées. J. Bot. 18: 134–140, 141–152, 380–385; 19: 63–68.

Nixon, K. C. 1989. Origins of Fagaceae. Pp. 23–44 *in* P. R. Crane & S. Blackmore (editors), Evolution, Systematics and Fossil History of the Hamamelidae, Vol. 2. 'Higher' Hamamelidae. Syst. Assoc. Special Vol. 40B. Clarendon Press, Oxford.

——— & J. M. Carpenter. 1996. On simultaneous analysis. Cladistics 12: 221–241.

Oginuma, K. 1999. Karyomorphology and evolution in the Juglandales: A review. Acta Phytotax. Geobot. 50: 229–241. [In Japanese with English summary and titles for figures and tables.]

——— & H. Tobe. 1992. Karyomorphology of Juglandaceae. Pp. 171–179 *in* R. Tanaka (editor), Plant Chromosome Research. Proc. Second Sino-Jap. Symp. Pl. Chromosomes.

———, Z. Gu & Z.-S. Yue. 1995. Karyomorphology of *Rhoiptelea* (Rhoipteleaceae). Acta Phytotax. Geobot. 46: 147–151.

Olmstead, R. G. & J. A. Sweere. 1994. Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. Syst. Biol. 43: 467–481.

Qiu, Y.-L., M. W. Chase, D. H. Les & C. R. Parks. 1993. Molecular phylogenetics of the Magnoliidae: Cladistic analyses of nucleotide sequences of the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 587–606.

———, ———, S. B. Hoot, E. Conti, P. R. Crane, K. J. Sytsma & C. R. Parks. 1998. Phylogenetics of the Hamamelidae and their allies: Parsimony analyses of nucleotide sequences of the plastid gene *rbc*L. Int. J. Pl. Sci. 159: 891–905.

Reveal, J. L. 1983. The Demoulin rule and newly mandated combinations in *Eriogonum* (Polygonaceae). Taxon 32: 292–295.

——— & A. B. Doweld. 1999. Validation of some suprageneric names in Magnoliophyta. Novon 9: 549–553.

Rogers, C. M. 1972. The taxonomic significance of the fatty acid content of seeds of *Linum*. Brittonia 24: 415–419.

Rzedowski, J. & R. Palacios-Chávez. 1977. El bosque de *Engelhardtia* (*Oreomunnea*) *mexicana* en la region de la Chinantla (Oaxaca, Mexico)—Una reliquia del Cenozoico. Bol. Soc. Bot. México 36: 93–123 + 4 plates.

Schaarschmidt, H. 1985. The relationship between *Carya* and *Platycarya* (Juglandaceae) and the natural classification of the family. Feddes Repert. 96: 345–361. [In German with English summary.]

Shorland, F. B. 1963. The distribution of fatty acids in plant lipids. Pp. 253–311 *in* T. Swain (editor), Chemical Plant Taxonomy. Academic Press, London.

Slabas, A. R., K. Elborough, D. Chase, B. Sinden, J. M. Martinez-Rivas, A. P. Brown, R. Swinhoe, J. W. Simon & T. Fawcett. 1993. Fatty acid synthesis in oilseeds. Pp. 81–95 *in* P. R. Shewry & K. Stobart (editors), Seed Storage Compounds. Clarendon Press, Oxford.

Smith, J. F. & J. J. Doyle. 1995. A cladistic analysis of chloroplast DNA restriction site variation and morphology for the genera of the Juglandaceae. Amer. J. Bot. 82: 1163–1172.

Soltis, D. E., P. S. Soltis, M. E. Mort, M. W. Chase, V. Savolainen, S. B. Hoot & C. M. Morton. 1998. Inferring complex phylogenies using parsimony: An empirical approach using three large DNA data sets for angiosperms. Syst. Biol. 47: 32–42.

Stanford, A. M., R. Harden & C. R. Parks. 2000. Phylogeny and biogeography of *Juglans* (Juglandaceae) based on *mat*K and ITS sequence data. Amer. J. Bot. 87: 872–882.

Stebbins, G. L. 1974. Flowering Plants: Evolution above the Species Level. Belknap Press, Cambridge, Massachusetts.

Stern, W. L. 1973. Development of the amentiferous concept. Brittonia 25: 316–333.

Stone, D. E. 1970. Evolution of cotyledonary and nodal vasculature in the Juglandaceae. Amer. J. Bot. 57: 1219–1225.

———. 1972. New World Juglandaceae, III. A new perspective of the tropical members with winged fruits. Ann. Missouri Bot. Gard. 59: 297–321.

———. 1973. Patterns in the evolution of amentiferous fruits. Brittonia 25: 371–384.

———. 1977. Juglandaceae. Pp. 28–53 *in* W. Burger (editor), Flora Costaricensis. Fieldiana, Bot., Vol. 40.

———. 1989. Biology and evolution of temperate and tropical Juglandaceae. Pp. 117–145 *in* P. R. Crane & S. Blackmore (editors), Evolution, Systematics, and Fossil History of the Hamamelidae, Vol. 2. 'Higher' Hamamelidae. Syst. Assoc. Special Vol. 40B. Clarendon Press, Oxford.

———. 1993. Juglandaceae. Pp. 348–359 *in* K. Kubitzki, J. G. Rohwer & V. Bittrich (editors), The Families and Genera of Vascular Plants, Vol. II. Flowering Plants—Dicotyledons. Springer-Verlag, Berlin.

———. 1997. Juglandaceae A. Richard ex Kunth. Pp. 416–428 *in* Flora of North America Editorial Committee (editors), Flora of North America, Vol. 3. Magnoliophyta: Magnoliidae and Hamamelidae. Oxford Univ. Press, New York.

——— & C. R. Broome. 1971. Pollen ultrastructure: Evidence for relationship of the Juglandaceae and the Rhoipteleaceae. Pollen & Spores 13: 5–14.

——— & ———. 1975. Juglandaceae A. Rich. ex Kunth. Pp. 1–35 *in* S. Nilsson (editor), World Pollen and Spore Flora, Vol. 4. Almqvist and Wiksell, Stockholm.

———, G. A. Adrouny & S. Adrouny. 1965. Morphological and chemical evidence on the hybrid nature of bitter pecan, *Carya* ×*lecontei*. Brittonia 17: 97–106.

———, ——— & R. H. Flake. 1969. New World Juglandaceae. II. Hickory nut oils, phenetic similarities, and evolutionary implications in the genus *Carya*. Amer. J. Bot. 56: 928–935.

———, J. Reich & S. Whitfield. 1964. Fine structure of the walls of *Juglans* and *Carya* pollen. Pollen & Spores 6: 379–392.

Su, S.-W. & J.-Q. He. 1984. Discovery of bisexual flowers in *Pterocarya stenoptera* C.DC. Acta Phytotax. Sin. 22: 256–258 [In Chinese with English abstract.]

Swofford, D. L. 2000. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), Vers. 4.0. Sinauer, Sunderland, Massachusetts.

Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for the amplification of three non-coding regions of chloroplast DNA. Pl. Molec. Biol. 17: 1105–1109.

Takhtajan, A. 1968. Classification and phylogeny with special reference to the flowering plants. Proc. Linn. Soc. London 179: 221–227.

———. 1980. Outline of the classification of flowering plants (Magnoliophyta). Bot. Rev. 46: 225–359.

———. 1997. Diversity and Classification of Flowering Plants. Columbia Univ. Press, New York.

Tang, Y. 1932. Timber anatomy of Rhoipteleaceae. Bull. Fan Mem. Inst. Biol. 3: 127–130.

Thorne, R. F. 1973. The 'Amentiferae' or Hamamelidae as an artificial group, a summary statement. Brittonia 25: 395–405.

———. 1983. Proposed new realignments in the angiosperms. Nordic J. Bot. 3: 85–117.

———. 1992. Classification and geography of the flowering plants. Bot. Rev. 58: 225–348.

Wainio, W. W. & E. B. Forbes. 1941. The chemical composition of forest fruits and nuts from Pennsylvania. J. Agric. Res. 62: 627–635.

Walker, J. W. & J. A. Doyle. 1975. The bases of angiosperm phylogeny: Palynology. Ann. Missouri Bot. Gard. 62: 664–723.

Whitehead, D. R. 1963. Pollen morphology in the Juglandaceae, I: Pollen size and pore number variation. J. Arnold Arbor. 46: 101–110.

———. 1965. Pollen morphology in the Juglandaceae, II: Survey of the family. J. Arnold Arbor. 46: 369–410.

Wing, S. L. & L. J. Hickey. 1984. The *Platycarya* perplex and the evolution of the Juglandaceae. Amer. J. Bot. 71: 388–411.

Withner, C. L. 1941. Stem anatomy and phylogeny of the Rhoipteleaceae. Amer. J. Bot. 28: 872–878.

Wolfe, J. A. 1973. Fossil forms of Amentiferae. Brittonia 25: 334–355.

———. 1978. A paleobotanical interpretation of Tertiary climates in the Northern Hemisphere. Amer. Sci. 66: 694–703.

———. 1995. Paleoclimatic estimates from Tertiary leaf assemblages. Ann. Rev. Earth Planet Sci. 23: 119–142.

Wu, C.-Y. & K. Kubitzki. 1993. Rhoipteleaceae. Pp. 584–585 *in* K. Kubitzki, J. G. Rohwer & V. Bittrich

(editors), The Families and Genera of Vascular Plants, Vol. II. Flowering Plants—Dicotyledons. Springer-Verlag, Berlin.

Wu, Z.-M. 1995. Cytological studies on some plants of woody flora in Huangshan, Anhui Province. J. Wuhan Bot. Res. 13: 107–112.

Zavada, M. S. & D. L. Dilcher. 1986. Comparative pollen morphology and its relationship to phylogeny of pollen in the Hamamelidae. Ann. Missouri Bot. Gard. 73: 348–381.

Zhang, Z.-Y., A.-M. Lu & J. Wen. 1994. Embryology of *Rhoiptelea chiliantha* (Rhoipteleaceae) and its systematic relationship. Cathaya 6: 57–66.

Zhou, R.-H. & Z.-H. Jiang. 1990. Chemical constituents of the Hamamelidae and their systematic significance. Cathaya 2: 63–76.

THE UTILITY OF MORPHOLOGICAL CHARACTERS FOR INFERRING PHYLOGENY IN SCANDICEAE SUBTRIBE SCANDICINAE (APIACEAE)[1]

Krzysztof Spalik[2] *and Stephen R. Downie*[3]

Abstract

The utility of 44 morphological characters for phylogenetic inference was evaluated against cladograms inferred from nuclear ribosomal DNA internal transcribed spacer (ITS) sequences for 89 representatives of Apiaceae including 58 accessions of Scandiceae subtribe Scandicinae. Distance-based analyses of morphological data confirmed the monophyly of many clades delimited previously on the basis of ITS sequences. However, morphology failed to support the monophyly of Scandicinae and the close affinity between the basally branching *Conopodium* and *Athamanta* groups. The infrageneric divisions in *Anthriscus*, *Chaerophyllum*, and *Osmorhiza*, traditionally defined on the basis of life history, leaf division, indumentum, and inflorescence architecture, are incongruent with the molecular results as these morphological characters are quite homoplastic. Least homoplastic, and therefore useful for generic delimitation within Scandicinae, are fruit characters such as epidermis morphology and primary ridge shape. *Rhopalosciadium* and *Chaerophyllopsis*, traditionally placed in Scandicinae but not included in the ITS study, do not occur within the subtribe upon analysis of morphological data. The former has affinity to Scandiceae subtribe Torilidinae, whereas the latter allies with the apioid superclade. The following taxonomic changes are suggested: (1) *Krasnovia* and *Neoconopodium* are included in *Kozlovia*; (2) section *Cerefolium* is restored in *Anthriscus* for *A. cerefolium*; and (3) *Myrrhoides* is transferred back to *Chaerophyllum*, with four sections recognized within the latter.

Key words: Apiaceae, morphology, phylogeny, Scandiceae, Scandicinae, taxonomy, Umbelliferae.

The compound umbel is a trademark of subfamily Apioideae and is a feature by which the whole family Apiaceae has earned its traditional name Umbelliferae. Together with other morphological features, such as two one-seeded mericarps joined by a bifurcate carpophore and styles emerging from a more or less flattened floral disc or stylopodium, the compound umbel supports the monophyly of the subfamily and delimits it from the other two subfamilies, Saniculoideae and Hydrocotyloideae. Molecular data corroborate these morphological data in revealing that Apioideae are indeed monophyletic and a sister group to subfamily Saniculoideae (Plunkett et al., 1997; Downie et al., 1998).

Although morphology delimits subfamily Apioideae, it has proved unreliable in demarcating monophyletic groups within the subfamily, and all attempts to generate a useful classification system of umbellifers using this type of evidence have failed (Constance, 1971; Pimenov & Leonov, 1993). The systems of Apiaceae classification, including the most influential system of Drude (1898), are usually based on fruit characters, particularly its shape and compression, the characteristics of ribs, secretory canals, and indumentum, endocarp sclerification, shape of endosperm, and the distribution of calcium oxalate crystals (Koch, 1824; de Candolle, 1830; Tausch, 1834; Bentham, 1867; Drude, 1898; Calestani, 1905; Koso-Poljansky, 1916). The only exception is the classification of Cerceau-Larrival (1962), who underlined the importance of pollen morphology and vegetative features, especially the internal contour of the endexine and shape of the cotyledons. However, characters of the fruit and those used more recently for stomata, pollen, cotyledons, and secondary metabolites (Cerceau-Larrival, 1962, 1963, 1965, 1971; Guyot, 1971; Guy-

[1] The authors thank the curators of B, BC, BM, E, ILL, KRA, KRAM, L, MO, P, W, and WA for loans of specimens; Alexei A. Oskolski and Vera M. Vinogradova for herbarium assistance; Charles E. Jarvis for checking Linnaean types; and Jean-Pierre Reduron, Mark F. Watson, Deborah S. Katz-Downie, Ronald L. Hartman, Victoria C. Hollowell, and two anonymous reviewers for helpful comments on the manuscript. This work was supported by grants from the Polish Committee for Scientific Research (KBN 6 P04C 02611) and the National Science Foundation (DEB-9407712).

[2] Department of Plant Systematics and Geography, Warsaw University, Aleje Ujazdowskie 4, 00-478 Warszawa, Poland. kspalik@ibb.waw.pl.

[3] Department of Plant Biology, University of Illinois, Urbana, Illinois 61801, U.S.A.

Table 1. Genera included in Scandiceae subtribe Scandicinae based on phylogenetic analysis of nuclear rDNA ITS sequences (Downie et al., 2000a).

Genus	No. of species	Distribution (center)
Anthriscus Pers.	9[a,c]	Eurasia/Africa (NE Mediterranean/Caucasus)
Athamanta L.	5[b]	Europe/N Africa (Italy and Balkans)
Balansaea Boiss. & Reut.	1	SW Mediterranean
Chaerophyllum L.	34[c]	Eurasia/N Africa/N America (Mediterranean/Caucasus)
Conopodium W. D. J. Koch	5[d]	Europe/N Africa (W Mediterranean)
Geocaryum Coss.	3[e]–15[f]	NE Mediterranean (Balkans)
Kozlovia Lipsky	1	Irano-Turanian
Krasnovia Popov ex Schischk.	1	Central Asia
Myrrhis Mill.	1	Central Europe
Myrrhoides Fabr.	1	SE Europe/W Asia
Neoconopodium Pimenov & Kljuykov	2[g]	Irano-Turanian/Himalaya
Osmorhiza Raf.	10[c,h]	Asia/America (N America)
Scandix L.	5–20[i]	Eurasia/Africa (E Mediterranean)
Sphallerocarpus DC.	1	East Asia
Tinguarra Parl.	2[j]	Canaries
Todaroa Parl.[k]	1	Canaries

[a] Spalik (1997). [b] Based on Tutin (1968), but excluding *A. macedonica* and *A. della-cellae*. [c] Table 2. [d] Silvestre (1973). [e] Ball (1968). [f] Engstrand (1977). [g] Pimenov & Kljuykov (1987). [h] Lowry & Jones (1984). [i] Pimenov & Leonov (1993). [j] *T. cervariifolia* and *T. montana*, excluding *T. sicula* transferred back to *Athamanta*. [k] Included based on Downie et al. (2000c).

ot et al., 1980; Harborne, 1971; Nielsen, 1971; Crowden et al., 1969; Harborne et al., 1969; Harborne & Williams, 1972) are homoplastic when optimized onto phylogenies inferred from molecular data (Plunkett et al., 1996; Katz-Downie et al., 1999).

Contrary to those relationships implicit in traditional classifications based on morphology, evolutionary relationships among umbellifers estimated on the basis of various types of molecular markers are generally congruent. Several major lineages have been identified, seven of which have been formally recognized as tribes (Downie et al., 2000b). One tribe, Scandiceae Spreng., is further divided into three subtribes: Scandicinae Tausch, Daucinae Dumort., and Torilidinae Dumort. (Downie et al., 2000a). Although most of these lineages bear traditional tribal or subtribal names, only Echinophoreae and Scandicinae are equivalent to those taxa that have been long recognized under these names (Downie et al., 2000a, c). Moreover, it is difficult to find morphological synapomorphies defining these newly identified lineages (Downie et al., 2000b). It seems therefore that the task of reclassifying umbellifers, at least at higher taxonomic levels, may be accomplished based on molecular rather than on traditional taxonomic data. The latter are, however, crucial for the circumscription and identification of taxa. Evaluating the utility of morphological markers for phylogenetic inference at different taxonomic levels is therefore of great importance.

Subtribe Scandicinae encompasses 16 genera with up to 110 species, with 7 of these genera monotypic (Table 1). Members of Scandicinae are diversified with respect to habit, reproductive strategy, and life history, which makes them a model group to study evolutionary tendencies. Most taxa are perennial polycarpic hemicryptophytes; *Conopodium*, *Kozlovia*, *Krasnovia*, *Neoconopodium*, and *Geocaryum* are geophytes; and *Scandix*, *Myrrhoides*, and some species of *Anthriscus* and *Chaerophyllum* are annual therophytes ("true" annuals) or hemicryptophytes (winter annuals). The most ecologically diversified genera are *Anthriscus* and *Chaerophyllum*, whose members occur in both primary (montane forests, meadows, and screes) and secondary (ruderal) communities. Members of *Anthriscus*, *Chaerophyllum*, and *Osmorhiza*, although each united by a similar fruit morphology and anatomy, differ in habit and, as such, infrageneric taxa for each have been recognized (Table 2). Molecular analyses have confirmed that each of these genera is monophyletic but do not support their infrageneric divisions (Downie et al., 2000a). This suggests that, despite criticism, fruit morphology may be a source of taxonomically valuable characters whereas plant habit may not.

In this study, we evaluate the utility of morphological characters in delimiting monophyletic

Table 2. Infrageneric classification of *Anthriscus*, *Chaerophyllum*, and *Osmorhiza* tested in this study using molecular and morphological data. For brevity, taxonomic authorities are omitted if they are provided in Table 3. The source of taxonomic treatment is given in parentheses after each generic name. The treatment of *Chaerophyllum* is taken from Schischkin (1950b) with later changes. Series names enclosed in quotation marks are not validly published. Likewise, infrageneric assignments of Koso-Poljansky (1916) are problematic, lacking designated types.

Anthriscus Pers. (Spalik, 1997)
section ***Anthriscus***
A. caucalis, *A. cerefolium*, *A tenerrima* Boiss. & Spruner
section ***Caroides*** Boiss.
A. kotschyi, *A. ruprechtii* Boiss.
section ***Cacosciadium*** (Rchb.) Neilr.
A. lamprocarpa, *A. nitida*, *A. schmalhausenii*, *A. sylvestris* [subsp. *sylvestris*, subsp. *nemorosa*, subsp. *fumarioides*, subsp. *alpina* (Vill.) Gremli]
Chaerophyllum L. (Schischkin, 1950b; Hedge & Lamond, 1972a, 1987; Czerepanov, 1995)
Subgenus ***Chaerophyllum***
series ***Aromatica*** Koso-Pol.
C. aromaticum, *C. aureum*
series ***Hirsuta*** Koso-Pol.
C. hirsutum
series ***Humilia*** Koso-Pol.
C. humile Stev.
"series ***Rosea*** Schischk."
C. roseum M. Bieb., *C. rubellum* Albov
"series ***Involucrata*** Schischk."
C. astrantiae, *C. borodinii*[a] Albov, *C. khorassanicum*
"series ***Temula*** Schischk."
C. temulum
Subgenus ***Golenkinianthe*** (Koso-Pol.) Schischk.
C. macrospermum
Subgenus ***Buniomorpha*** Koso-Pol.
series ***Angelicifolia*** Koso-Pol.
C. angelicifolium[a] M. Bieb., *C. confusum* Woron. ex Grossh., *C. meyeri*
series ***Crinita*** Koso-Pol.
C. crinitum
series ***Bulbosa*** Koso-Pol.
C. bulbosum
Species not considered by Schischkin (1950b):
C. atlanticum, *C. azoricum*, *C. byzantinum*, *C. coloratum* L., *C. creticum* Boiss. & Heldr., *C. elegans*, *C. hakkiaricum* Hedge & Lamond, *C. heldreichii* Orph. ex Boiss., *C. leucolaenum* Boiss., *C. libanoticum*, *C. macropodum*, *C. magellense*, *C. nivale* Hedge & Lamond, *C. procumbens*, *C. reflexum* Lindl., *C. tainturieri*, *C. villarsii*, *C. villosum* DC.
Osmorhiza Raf. (Lowry & Jones, 1984)
Subgenus ***Osmorhiza***
section ***Osmorhiza***
O. aristata, *O. claytonii*, *O. longistylis*
section ***Mexicanae*** Constance & Shan ex Lowry & A. G. Jones
O. mexicana [subsp. *mexicana*, subsp. *bipatriata*], *O. brachypoda*, *O. glabrata* Phil.

Table 2. Continued.

section ***Nudae*** Constance & Shan ex Lowry & A. G. Jones
O. berteroi, *O. depauperata*, *O. purpurea*
Subgenus ***Glycosma*** (Nutt.) Drude
O. occidentalis

[a] Original spellings *angelicaefolium* and *borodini* are contrary to Art. 60.8 and Art. 60.11 of the Botanical Code (Greuter et al., 2000), respectively.

groups within Scandiceae subtribe Scandicinae in light of the results of our recent molecular systematic study of the group using nuclear ribosomal DNA ITS sequences (Downie et al., 2000a). We are particularly interested in identifying those morphological characters that are most useful in delimiting genera and infrageneric taxa. Similarly, we seek to provide a new circumscription of the subtribe based on morphology which would permit identification of members of this clade. We also confirm the position of two monotypic genera previously placed in Scandicinae, *Rhopalosciadium* Rech. f. and *Chaerophyllopsis* H. Boissieu, that were not available for our earlier ITS study due to a lack of sufficient material.

Materials and Methods

Taxon Sampling

The taxa examined were chosen to achieve nearly complete overlap between morphological and ITS data matrices. In total, 91 taxa were analyzed, with 89 taxa included in both matrices. The ingroup, or subtribe Scandicinae, was represented by 58 accessions belonging to 15 genera. The monotypic *Todaroa* was not included as its affinity to Scandicinae has only recently been confirmed (Downie et al., 2000c). *Anthriscus*, *Chaerophyllum*, and *Osmorhiza* were broadly sampled in order to verify their infrageneric divisions. Subtribes Daucinae and Torilidinae, forming close outgroups, were represented by six and three species, respectively. Additional outgroups represented other major lineages of umbellifers or encompassed taxa that had been previously excluded from Scandicinae based on molecular data, i.e., *Grammosciadium*, *Rhabdosciadium*, and *Bubon*. The genus *Bubon*, formerly recognized in *Athamanta*, was recently reinstated (Downie et al., 2000a). Two species included in the morphological analysis, *Rhopalosciadium stereocalyx* Rech. f. and *Chaerophyllopsis huaui* H. Boissieu, were not sampled in our ITS study (Downie et al., 2000a). The first species is known only from the type gathering, and we have not found any good quality material of the second one.

MOLECULAR DATA

We considered 89 of the 134 accessions from our prior study (Downie et al., 2000a; Table 3), with no additional sequencing of the ITS region undertaken as part of this study. Initially, ITS1 and ITS2 sequences were aligned using the program CLUSTAL V (Higgins et al., 1992) and manually adjusted where necessary. The sequences of the intervening 5.8S subunit were not included as they were incomplete for several taxa and those that were available were not variable enough to justify their inclusion or additional sequencing. GenBank accession numbers of separate ITS1 and ITS2 sequences are provided in Table 3. The aligned matrix is available upon request from either author.

MORPHOLOGICAL DATA

Herbarium material was obtained from the following institutions: B, BC, BM, E, ILL, KRA, KRAM, L, MO, P, W, WA (abbreviations according to Holmgren et al., 1990). Specimen identifications were verified using several keys, the most important being *Flora Europaea* (Tutin et al., 1968), *Flora of Turkey* (Davis, 1972), *Flora Iranica* (Hedge et al., 1987), and *Flora of the USSR* (Schischkin, 1950a). For *Geocaryum*, *Osmorhiza*, and *Anthriscus*, their respective revisions were used (Engstrand, 1977; Lowry & Jones, 1984; Spalik, 1997).

The morphological matrix comprised 44 discrete characters, including 24 binary, 9 multistate and unordered, and 11 ordinal (Appendices 1 and 2). As ordinal we declared only those characters that can be unambiguously ordered (i.e., those that either coded measurements or, for three-state characters, those in which one state was apparently intermediate between the other two). Chosen characters represented life history and vegetative morphology (1–6), bract and bracteole morphology (7–13), inflorescence architecture (14–20), floral morphology (21–26), and fruit morphology (27–44). Character polymorphisms and uncertainties were distinguished using the "()" versus "{}" coding options available in PAUP* 4.0 (Swofford, 1998).

PHYLOGENETIC ANALYSES

For maximum parsimony analysis of ITS sequences, gaps were treated as missing data and character states were assumed unordered. Heuristic searches, implemented using PAUP* 4.0, employed tree bisection-reconnection (TBR) branch swapping with options MULPARS and STEEPEST DESCENT selected. To locate possible islands of most parsimonious trees, 100 heuristic searches were initiated, saving only five shortest trees from each search. These trees were then used as starting trees for TBR branch swapping. The search was stopped when the number of trees reached the memory limit of 16,000. The strict consensus tree was used as a topological constraint in a subsequent search using the inverse constraint method of Catalán et al. (1997). This time, 1000 heuristic searches were initiated saving only those trees that did not fit the constraint tree (no more than two trees per replicate were saved). Because all trees found were longer than those most parsimonious, this then suggested that the strict consensus tree summarizes all possible topologies of trees at that length, even though their exact number is not known.

Distance trees were obtained from neighbor-joining analyses using PAUP* 4.0, estimated using Kimura's (1980) two- and three-parameter, Jukes-Cantor, and maximum likelihood distance measures. Heuristic searches using the criterion of minimum evolution were also carried out using neighbor-joining trees and a variety of distance measures. Bootstrap analyses were performed using 100 resampled data sets, with 50 best trees per replicate saved.

Morphological data were analyzed using both maximum parsimony and distance methods available in PAUP* 4.0. For the maximum parsimony analysis, TBR branch swapping, with MULPARS and STEEPEST DESCENT, was employed. One hundred heuristic searches were initiated, saving only five shortest trees from each search. These trees were used as starting trees for TBR branch swapping. The search was terminated when the number of trees reached the memory limit of 16,000. Distance trees were constructed using neighbor-joining and minimum evolution methods using mean and total character differences.

In the analysis of the combined ITS and morphological data set, we included morphological data for *Rhopalosciadium stereocalyx* and *Chaerophyllopsis huaui*. Because these species were not included in our prior study (Downie et al., 2000a), ITS data were not available and therefore these characters were scored as missing. For parsimony analysis, the search strategy was the same as for the separate analysis of ITS data. Distance methods comprised neighbor-joining and minimum evolution with mean and total character differences selected.

EVOLUTION OF MORPHOLOGICAL CHARACTERS

To evaluate the utility of morphological characters in delimiting monophyletic groups and to assess the degree of homoplasy in these characters,

Table 3. Accessions of Apiaceae examined for nuclear rDNA ITS sequence variation. Material of uncertain wild origin obtained from botanical gardens is denoted as cultivated. Collection data, locations of voucher specimens, and details on the DNA sequencing are provided elsewhere: (1) Downie and Katz-Downie (1996); (2) Downie et al. (1998); (3) Downie et al. (2000a).

Taxon	Origin	Reference	GenBank accession no.	
			ITS1	ITS2
Anthriscus caucalis M. Bieb.	France (cult.)	2	U79601	U79602
Anthriscus cerefolium (L.) Hoffm.	Spain (cult.)	1	U30532	U30533
Anthriscus kotschyi Boiss. & Balansa	Turkey	3	AF073579	AF073580
Anthriscus lamprocarpa Boiss.	Jordan	3	AF073581	AF073582
Anthriscus nitida (Wahlenb.) Hazsl.	France	3	AF073595	AF073596
Anthriscus schmalhausenii (Albov) Koso-Pol.	Russia	3	AF073587	AF073588
Anthriscus sylvestris (L.) Hoffm. subsp. *sylvestris*	Russia (cult.)	2	U79603	U79604
Anthriscus sylvestris subsp. *fumarioides* (Waldst. & Kit.) Spalik	Yugoslavia [now Serbia]	3	AF073575	AF073576
Anthriscus sylvestris subsp. *nemorosa* (M. Bieb.) Koso-Pol.	Turkey	3	AF073573	AF073574
Apium graveolens L.	France (cult.)	1	U30552	U30553
Athamanta cretensis L.	France	3	AF073685	AF073686
Athamanta della-cellae Asch. & Barbey ex E. A. Durand & Barratte	Libya	3	AF073565	AF073566
Athamanta sicula L.	Morocco	3	AF073683	AF073684
Athamanta turbith (L.) Broth. subsp. *turbith*	Slovenia	3	AF073687	AF073688
Balansaea glaberrima (Desf.) Maire	Algeria	3	AF073689	AF073690
Bubon macedonicum L. subsp. *macedonicum*	Greece	3	AF073541	AF073542
Bunium elegans (Fenzl) Freyn	Iran	3	AF073543	AF073544
Carum carvi L.	Finland (cult.)	2	U78377	U78437
Caucalis platycarpos L.	Germany (cult.)	2	U78364	U78424
Chaerophyllum aromaticum L.	Poland	3	AF073631	AF073632
Chaerophyllum astrantiae Boiss. & Balansa	Turkey	3	AF073653	AF073654
Chaerophyllum atlanticum Coss.	Morocco	3	AF073633	AF073634
Chaerophyllum aureum L.	Turkey	3	AF073655	AF073656
Chaerophyllum azoricum Trel.	Spain	3	AF073657	AF073658
Chaerophyllum bulbosum L.	Turkey	3	AF073659	AF073660
Chaerophyllum byzantinum Boiss.	Turkey	3	AF073635	AF073636
Chaerophyllum crinitum Boiss.	Iran	3	AF073661	AF073662
Chaerophyllum elegans Gaudin	Switzerland	3	AF073663	AF073664
Chaerophyllum hirsutum L.	France	3	AF073665	AF073666
Chaerophyllum khorassanicum Czerniak. ex Schischk.	Turkmenistan	2	U78366	U78426
Chaerophyllum libanoticum Boiss. & Kotschy	Turkey	3	AF073637	AF073638
Chaerophyllum macropodum Boiss.	Iran	3	AF073671	AF073672
Chaerophyllum macrospermum (Willd. ex Spreng.) Fisch. & C. A. Mey.	Turkey	3	AF073651	AF073652

Table 3. Continued.

Taxon	Origin	Reference	GenBank accession no.	
			ITS1	ITS2
Chaerophyllum magellense Ten.	Italy	3	AF073669	AF073670
Chaerophyllum meyeri Boiss. & Buhse	Iran	3	AF073639	AF073640
Chaerophyllum procumbens (L.) Crantz	U.S.A., Indiana	3	AF073643	AF073644
Chaerophyllum tainturieri Hook. & Arn.	U.S.A., Arkansas	3	AF073645	AF073646
Chaerophyllum temulum L.	Poland	3	AF073641	AF073642
Chaerophyllum villarsii W. D. J. Koch	Switzerland	3	AF073667	AF073668
Chaetosciadium trichospermum (L.) Boiss.	Jordan	2	U78363	U78423
Cicuta virosa L.	Finland (cult.)	2	U78372	U78432
Conopodium ramosum Costa	Portugal	3	AF073693	AF073694
Daucus carota L.	Germany (cult.)	1	U27589	U30315
Echinophora tenuifolia L. subsp. *sibthorpiana* (Guss.) Tutin	Turkey	3	AF073529	AF073530
Elaeosticta allioides (Regel & Schmalh.) Kljuykov, Pimenov & V. N. Tikhom.	Turkmenistan	3	AF073547	AF073548
Falcaria vulgaris Bernh.	Russia, Rostov prov.	2	U78378	U78438
Geocaryum macrocarpum (Boiss. & Spruner) Engstrand	Greece	3	AF073607	AF073608
Grammosciadium daucoides DC.	Turkey	3	AF073559	AF073560
Grammosciadium macrodon Boiss.	Turkey	3	AF073553	AF073554
Grammosciadium platycarpum Boiss. & Hausskn.	Turkey	3	AF073551	AF073552
Grammosciadium pterocarpum Boiss.	Turkey	3	AF073557	AF073558
Heracleum sphondylium L.	Finland (cult.)	1	U30544	U30545
Heteromorpha arborescens (Spreng.) Cham. & Schltdl.	Africa (cult. Spain)	1	U27578	U30314
Kozlovia paleacea (Regel & Schm.) Lipsky	Afghanistan	3	AF073597	AF073598
Krasnovia longiloba (Kar. & Kir.) Popov ex Schischk.	Kazakhstan	3	AF073599	AF073600
Laserpitium hispidium M. Bieb.	Russia, Krasnodar prov.	2	U78361	U78421
Laserpitium petrophilum Boiss. & Heldr.	Turkey	3	AF073567	AF073568
Lecokia cretica (Lam.) DC.	Jordan	2	U78358	U78418
Myrrhis odorata (L.) Scop.	Europe (cult. U.S.A.)	1	U30530	U30531
Myrrhoides nodosa (L.) Cannon	Armenia	3	AF073675	AF073676
Neoconopodium capnoides (Decne.) Pimenov & Kljuykov	Himalaya	3	AF073601	AF073602
Neoconopodium laseroides (Hedge & Lamond) Pimenov & Kljuykov	Afghanistan	3	AF073603	AF073604
Orlaya grandiflora (L.) Hoffm.	France (cult.)	1	U30524	U30525

Table 3. Continued.

Taxon	Origin	Reference	GenBank accession no.	
			ITS1	ITS2
Osmorhiza aristata (Thunb.) Rydb.	Japan	3	AF073609	AF073610
Osmorhiza berteroi DC.	U.S.A., California	2	U78365	U78425
Osmorhiza brachypoda Torr.	U.S.A., California	3	AF073617	AF073618
Osmorhiza claytonii (Michx.) C. B. Clarke	U.S.A., Indiana	3	AF073615	AF073616
Osmorhiza depauperata Phil.	U.S.A., Montana	3	AF073611	AF073612
Osmorhiza longistylis (Torr.) DC.	U.S.A., Illinois	2	U79617	U79618
Osmorhiza mexicana subsp. *bipatriata* (Constance & Shan) Lowry & A. G. Jones	Mexico	3	AF073623	AF073624
Osmorhiza mexicana Griseb. subsp. *mexicana*	Mexico	3	AF073621	AF073622
Osmorhiza occidentalis (Nutt.) Torr.	U.S.A., Wyoming	2	U79619	U79620
Osmorhiza purpurea (J. M. Coult. & Rose) Suksd.	U.S.A., Oregon	3	AF073625	AF073626
Pastinaca sativa L.	Germany (cult.)	1	U30546	U30547
Petroselinum crispum (Mill.) A. W. Hill	Germany (cult.)	2	U78387	U78447
Pimpinella peregrina L.	Germany (cult.)	1	U30592	U30593
Pseudorlaya pumila (L.) Grande	Germany (cult.)	1	U30522	U30523
Rhabdosciadium aucheri Boiss.	Iran	3	AF073549	AF073550
Scaligeria moreana Engstrand	Greece	3	AF073545	AF073546
Scandix balansae Reut. ex Boiss.	Germany (cult.)	2	U79621	U79622
Scandix iberica M. Bieb.	Jordan	3	AF073627	AF073628
Scandix pecten-veneris L.	Germany (cult.)	1	U30538	U30539
Scandix stellata Banks & Sol.	Jordan	3	AF073629	AF073630
Sium latifolium L.	Hungary (cult.)	2	U78370	U78430
Smyrnium olusatrum L.	France (cult.)	1	U30594	U30595
Sphallerocarpus gracilis (Bess. ex Trevir.) Koso-Pol.	Russia, Siberia	3	AF073677	AF073678
Tinguarra cervariifolia (DC.) Parl.	Spain, Canaries	3	AF073681	AF073682
Tinguarra montana (Webb ex H. Christ) A. Hansen & G. Kunkel	Spain, Canaries	3	AF073679	AF073680
Torilis nodosa (L.) Gaertn.	France (cult.)	1	U30534	U30535

Table 4. Sequence characteristics of the two nuclear rDNA internal transcribed spacers, separately and combined, for 89 representatives of Apiaceae subfamily Apioideae including 58 accessions of Scandiceae subtribe Scandicinae.

Sequence characteristic	ITS1	ITS2	ITS1 & ITS2
Nucleotide sites			
Spacer length variation (bp)	206–221	203–229	421–444
No. of total aligned positions	249	243	492
No. (and %) of aligned positions ambiguous	10 (4)	6 (2)	16 (3)
No. (and %) of aligned positions constant	64 (26)	41 (17)	105 (21)
No. (and %) of aligned positions autapomorphic	30 (12)	33 (14)	63 (13)
No. (and %) of aligned positions parsimony informative	145 (58)	163 (67)	308 (63)
Sequence divergence (mean and range in %)			
All accessions	18.4 (0–32.8)	19.4 (0–37.3)	19.0 (0–33.6)
Subtribe Scandicinae only	11.8 (0–22.9)	12.5 (0–23.1)	12.2 (0–22.0)

we optimized them onto all ITS-derived trees (whether maximum parsimony or distance) using MacClade 3.07 (Maddison & Maddison, 1992). The tree that best explained the variation in morphology was used to infer the number of evolutionary steps and consistency (CI), retention (RI), and rescaled consistency (RC) indices of these characters. To identify those characters that are most useful for the definition of formally recognized taxa, and of genera in particular, we considered character state changes on the strict consensus tree resulting from parsimony analysis of the combined data set.

Results

Sequence analysis

Due to the exclusion of some Scandicinae and outgroup taxa, the length of the aligned ITS sequences was shorter and the number of ambiguous sites lower than those values presented in our earlier study (Downie et al., 2000a). In this analysis of 89 taxa, the aligned matrix had 492 characters, representing 249 positions from ITS1 and 243 positions from ITS2. The alignment of 10 positions in ITS1 and 6 positions in ITS2 were ambiguous, so these regions were excluded from the analysis. The numbers of constant, autapomorphic, and parsimony-informative positions were similar for both spacers (Table 4), and the ratio of terminal taxa (89) to informative characters across both spacers (308) was 1:3.5. Mean sequence divergence values were also similar for both spacers (18.4 and 19.4% for ITS1 and ITS2, respectively), reaching a maximum of 33.6% pairwise sequence divergence for combined ITS1 and ITS2 data.

Phylogenetic analysis of ITS data

Maximum parsimony analysis of all 476 unambiguously aligned positions resulted in more than 16,000 minimal length trees, each 1746 steps long, with consistency indices of 0.404 and 0.376 (with and without uninformative characters, respectively), and a retention index of 0.748. Despite the high number of trees, the strict consensus tree is well resolved (Fig. 1). At the suprageneric level, most branches are supported by high bootstrap values. The neighbor-joining tree, calculated using Jukes-Cantor distance (Fig. 2), is similar to the parsimony strict consensus tree with few notable differences (discussed below). Trees of nearly identical topology were obtained using other distance measures (not shown).

All resultant parsimony and distance trees were generally congruent to those obtained using a broader sampling of Scandicinae and outgroup taxa (Downie et al., 2000a); the few differences observed comprise minor rearrangements of some weakly supported clades. All major groups are similar to those obtained previously and include four tribes (Scandiceae, Smyrnieae Spreng., Oenantheae Dumort., and Heteromorpheae M. F. Watson & S. R. Downie) and the "apioid superclade" (Downie et al., 2000b). Scandiceae are supported as monophyletic (with bootstrap values of 93% and 95%, in the neighbor-joining and parsimony analyses, respectively) and include three lineages equivalent to subtribes Scandicinae, Daucinae, and Torilidinae (Figs. 1, 2). Within Scandicinae, several additional clades are distinguished, corresponding to approximate generic and infrageneric categories (these informal groups are identified by double quotes in all tree figures presented herein).

Sister to all other Scandicinae is a clade comprising four genera: *Athamanta*, *Tinguarra*, *Conopodium*, and the monotypic *Balansaea* (which was synonymized with *Conopodium* by Engstrand, 1973; Figs. 1, 2). Two species formerly recognized in *Athamanta*—*A. macedonica* and *A. della-cel-*

lae—are excluded from this clade. The former is now treated as *Bubon macedonicum*, whereas the latter shows a close relationship with *Daucus* (Downie et al., 2000a). The monophyly of the clade formed by these four genera is strongly supported (with 100% bootstrap values); however, the phylogenetic relationships among its members are somewhat ambiguous. *Athamanta* and *Tinguarra* are each monophyletic and form a weakly supported clade (denoted as the *Athamanta* group) according to the neighbor-joining analysis (Fig. 2), but this relationship is not supported by maximum parsimony (Fig. 1). The *Conopodium* group (i.e., encompassing representatives of *Conopodium* sensu Engstrand, 1973) is not monophyletic.

Within Scandicinae proper, *Sphallerocarpus* forms an isolated branch and is variously placed in Figures 1 and 2. The *Chaerophyllum* group is well supported and also includes the monotypic *Myrrhoides*, although in some distance and maximum parsimony trees (not shown) *Myrrhoides* is sister to *Chaerophyllum*. Those members examined from *Chaerophyllum* form three subclades, denoted as the *C. aureum*, *C. hirsutum*, and *C. temulum* groups. These groups do not match the subgenera traditionally recognized in *Chaerophyllum* (Table 2), since the *C. aureum* clade includes representatives of subgenera *Chaerophyllum*, *Golenkinianthe*, and *Buniomorpha*, whereas members of the *C. hirsutum* and *C. temulum* clades are classified in subgenus *Chaerophyllum*. Of the nine series distinguished within *Chaerophyllum* (Schischkin, 1950b), five are monotypic. Of the remaining four, two series, "*Involucrata*" and "*Aromatica*," were each represented in our study by two species. These series are not supported by ITS data.

Seven genera of Scandicinae belong to a "crown" clade highly supported in both trees (Figs. 1, 2) that is sister to *Scandix*. The relationships among the members of this terminal clade, however, are ambiguous. *Anthriscus* and *Osmorhiza* are each monophyletic, although bootstrap values supporting the *Anthriscus* clade are less than 50%. The infrageneric classification of *Anthriscus* is only partly supported, as section *Anthriscus*, represented by *A. caucalis* and *A. cerefolium*, is not monophyletic. All included members of section *Cacosciadium* (Rchb.) Neilr. form a clade (Figs. 1, 2). Interestingly, Levantine *A. lamprocarpa*, represented by an accession from Jordan, is grouped with European *A. sylvestris* subsp. *sylvestris*, sampled from Russia, and not with the parapatric *A. sylvestris* subsp. *nemorosa*, represented in this study by an accession from Turkey. *Osmorhiza* includes three lineages, with *O. aristata*, the only Asiatic member of the genus, sister to all other *Osmorhiza* species. The two remaining members of section *Osmorhiza* (*O. claytonii* and *O. longistylis*) form the next branch, denoted as the *O. claytonii* group. The *O. berteroi* group comprises most of the species, including *O. occidentalis*, the sole member of subgenus *Glycosma*. This species, clearly distinct from other members of the genus with respect to its leaf division, flower color, and fruit morphology, is allied with *O. depauperata*, although with moderate bootstrap support (54% and 68%, in distance and parsimony analyses, respectively).

The monotypic *Myrrhis* from Central Europe is sister to East Mediterranean *Geocaryum*, although this relationship is not at all supported by high bootstrap values. The *Kozlovia* group is better supported (54% and 71% bootstrap values for distance and parsimony trees, respectively) and includes four Asiatic taxa: Irano-Turanian *Kozlovia*, Central Asiatic *Krasnovia*, and both species of Himalayan *Neoconopodium*.

PHYLOGENETIC ANALYSIS OF MORPHOLOGICAL DATA

Parsimony analysis of morphological data resulted in 9516 trees, each of length 427 steps, and consistency and retention indices of 0.183 and 0.699, respectively. Resolution of its consensus tree (not shown) is poor. *Anthriscus*, *Chaerophyllum*, *Geocaryum*, *Osmorhiza*, *Myrrhis*, *Myrrhoides*, *Scandix*, and the *Kozlovia* group formed a single polytomous clade, with only *Scandix* retained as monophyletic. Neighbor-joining trees (not shown) showed a similar pattern in which the same genera were grouped together, but the monophyly of some was not maintained. The tree obtained using total character differences and minimum evolution (Fig. 3) was most congruent with those trees inferred from ITS sequences. However, in this minimum evolution tree, neither Scandiceae nor any of its three subtribes are retained as monophyletic. The majority of Scandicinae form a clade that is sister to a group comprising representatives of Daucinae and Torilidinae (the latter nested within the former), the *Athamanta* group, the former members of *Athamanta* (*Bubon macedonicum* and *A. della-cellae*), and *Echinophora tenuifolia*. In the ITS analyses, *Bubon macedonicum* and *Echinophora tenuifolia* are placed in the apioid superclade (Figs. 1, 2).

Three genera of Scandiceae subtribe Scandicinae (*Sphallerocarpus*, *Conopodium*, and *Balansaea*), as circumscribed on the basis of molecular data, fall outside of the tribe when only morphology is considered. *Sphallerocarpus* is placed close to *Smyr-*

nium, the latter a representative of tribe Smyrnieae that in the distance analyses of ITS data is sister to Scandiceae. *Conopodium* and *Balansaea* form a separate clade with no near relatives. The close relationship between the *Athamanta* and *Conopodium* groups, as indicated by the molecular analyses, is not at all apparent.

Two species traditionally placed in Scandicinae that were not included in our earlier molecular analyses (Downie et al., 2000a), *Rhopalosciadium stereocalyx* and *Chaerophyllopsis huaui*, are also placed outside of the subtribe. The first is sister to the clade of *Torilis*, *Chaetosciadium*, and *Pseudorlaya*, all members of Scandiceae subtribes Torilidinae and Daucinae (Fig. 3), whereas the second is sister to *Petroselinum*, with this clade placed within the apioid superclade.

Phylogenetic analysis of morphological data (Fig. 3) shows that all genera of Scandicinae are monophyletic. However, many of the subclades identified within *Anthriscus*, *Chaerophyllum*, and *Osmorhiza* on the basis of ITS data (Figs. 1, 2) are not supported upon consideration of morphology. The only exception is the *C. hirsutum* group. None of the clades occurring within *Chaerophyllum* coincide with the subgenera recognized by Schischkin (1950b). In *Anthriscus*, neither section *Cacosciadium* nor section *Anthriscus* (including *A. caucalis* and *A. cerefolium*) are monophyletic. In *Osmorhiza*, morphology supports the isolated position of *O. occidentalis* (subg. *Glycosma*) and the monophyly of the subgenus and section *Osmorhiza*. All species retained in *Athamanta* by Downie et al. (2000a) are grouped together and placed close to *Tinguarra*; however, this clade also encompasses the former members of *Athamanta* (i.e., *Bubon macedonicum* and *A. della-cellae*).

PHYLOGENETIC ANALYSIS OF COMBINED DATA

Maximum parsimony analysis of combined ITS and morphological data resulted in 544 trees, each of length 2272 steps, consistency indices of 0.345 and 0.321 (with and without uninformative characters, respectively), and retention index of 0.717. The topology of their strict consensus tree (Fig. 4) is similar to that obtained using only molecular data (Fig. 1); notable differences include the positions of *Geocaryum* and *Myrrhis*, and of *Balansaea* and *Conopodium* (the *Conopodium* clade). In Figures 1 and 2, *Geocaryum* and *Myrrhis* are sister taxa, whereas in Figure 4 *Geocaryum* is sister to the *Kozlovia* group while *Myrrhis* is sister to *Osmorhiza*. The new addition *Rhopalosciadium* is sister to *Chaetosciadium* (Torilidinae), while the second novel taxon *Chaerophyllopsis* is placed outside of Scandiceae and is sister to *Apium*.

EVOLUTION OF MORPHOLOGICAL CHARACTERS

Of all the trees inferred from ITS data, the neighbor-joining trees obtained using Jukes-Cantor distance or total and mean character differences best explained the morphological variation. Their lengths, as estimated using only morphological data, were each 547 steps (with CI of 0.143 and RI of 0.588). When morphological characters were mapped onto the neighbor-joining trees obtained using Kimura's two- and three-parameter and Tajima-Nei distances, 548 steps were required; mapping these characters onto the maximum parsimony trees required from 549 to 572 steps. Not surprisingly, the tree inferred from analysis of combined ITS and morphological data better explained the variation in morphological characters than any of the trees inferred from ITS data alone. Here, upon the removal of *Chaerophyllopsis* and *Rhopalosciadium*, with no molecular data available for either, only 501 steps were required (with CI of 0.156 and RI of 0.629).

The number of steps and consistency, retention, and rescaled consistency indices of morphological characters estimated on the Jukes-Cantor/neighbor-joining tree inferred from ITS data are presented in Table 5. Most rescaled consistency index values are lower than 0.2, with only six characters having a value greater than 0.3. These six characters all represent fruit morphology, and include the presence/absence of a pedicel-like appendage (character no. 32, Appendix 1), the shape of the primary ridge (35), the presence/absence of secondary ridges (38), the appearance (42) and texture (43) of the cuticle, and the color of the epidermis (44). Among the most homoplastic characters are reproductive strategy (1) and leaf division (3). We point out here that these two characters are often diagnostic at infrageneric levels.

Those 11 characters that are most useful for delimiting certain lineages were mapped onto the consensus tree obtained from the analysis of combined data (Fig. 4). To facilitate mapping, the coding of three ordinal characters was simplified: states "incised" and "pinnate" in character 10 for bracteoles, states "reduced" and "absent" in character 21 for sepal presence, and states "obsolete" and "short" in character 34 for beak length were each combined. The polymorphism exhibited in character 36 for primary ridge indumentum was scored as an additional state. Based on morphology, neither Scandiceae nor any of its three subtribes can be

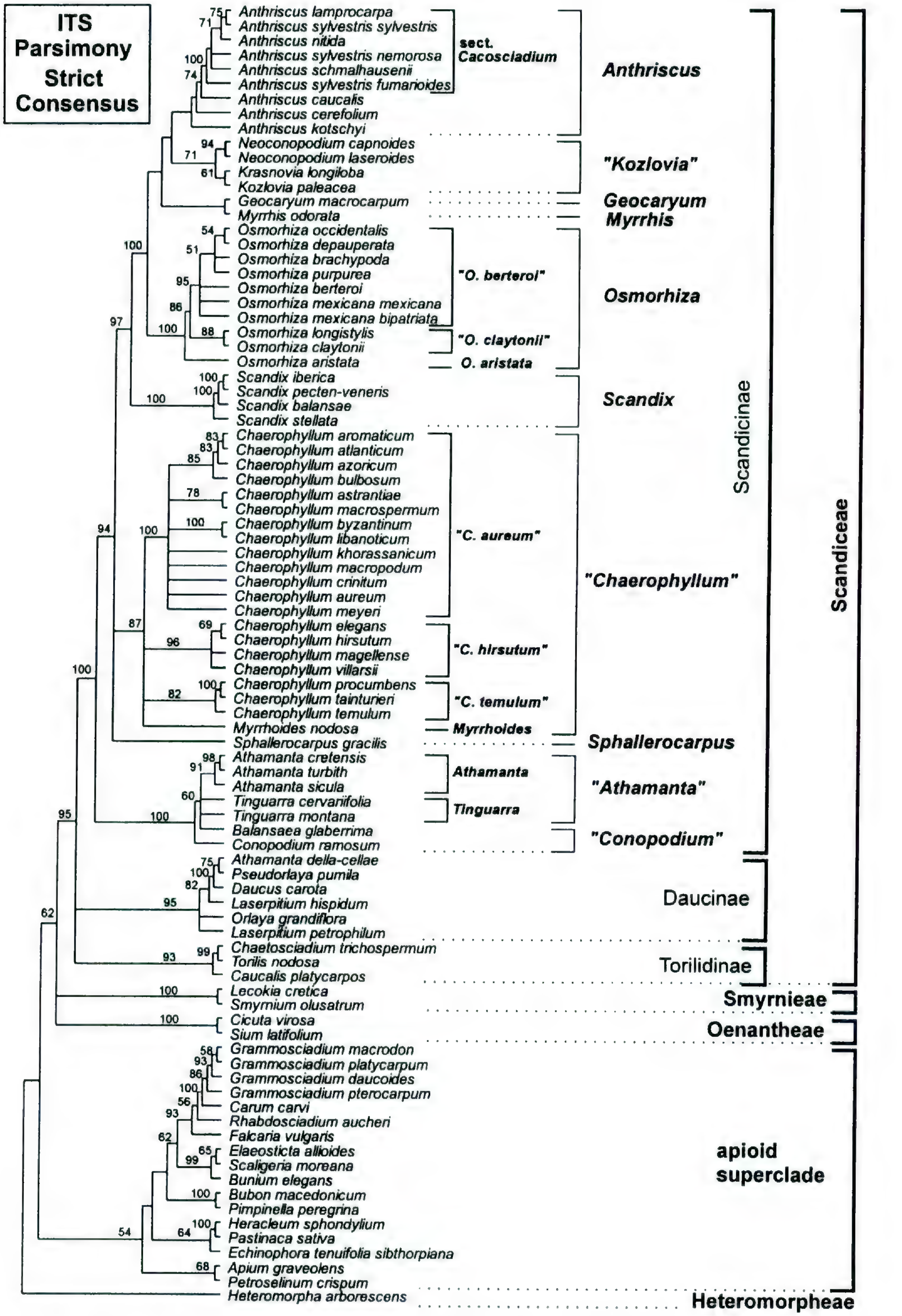


Figure 1. Strict consensus of 16,000 minimal length 1746-step trees derived from equally weighted maximum parsimony analysis of 89 nuclear rDNA ITS sequences from Scandiceae subtribe Scandicinae and outgroups (CI excluding uninformative characters = 0.376; RI = 0.748). Bootstrap values are indicated along respective nodes. Complete taxon names including ranks of infraspecific taxa are provided in Table 3. Bolder brackets at far right indicate

unambiguously defined. Most genera of Scandicinae have reduced sepals, and several genera are also characterized by an areolate fruit epidermis with a shiny aculeate cuticle. Secondary ridges appear twice, in Daucinae and in Torilidinae (Fig. 4); alternatively, they were gained in the common ancestor of Scandiceae and subsequently lost in Scandicinae (not shown). The genera of Scandicinae are usually well delimited although sometimes only on a set of homoplastic characters that may include plesiomorphies. For instance, the *Athamanta* group, which includes representatives of *Athamanta* and *Tinguarra*, is well separated from its sister *Conopodium* group, but only by those characters that have evolved in the latter (e.g., reduced sepals, no primary ridge indumentum, and globose tubers). *Chaerophyllum* may be defined by its broad rounded primary ribs; this character state is, however, homoplastic since it also appears in three species of *Scandix* and in *Carum* and *Grammosciadium*, the last two genera now placed in the apioid superclade, although *Grammosciadium* was once recognized in Scandiceae and regarded as closely related to *Chaerophyllum*. Ribs angular at top of fruit but obsolete below and deprived of distinct indumentum unambiguously delimit *Anthriscus*. A unique feature of *Osmorhiza* is a pedicel-like fruit appendage which is, however, lost in *O. occidentalis*. A long beak is synapomorphic for *Scandix*, which is also characterized by incised or pinnate bracteoles. Similar bracteole types also occur in *Daucus*, *Pseudorlaya*, and *Grammosciadium*. Apart from the *Conopodium* group, globose tubers also characterize the *Kozlovia* group and *Geocaryum*; outside Scandicinae, they are found in *Bunium* (and several other taxa not considered in this study).

Generally, we have not found any unambiguous character that would define subclades in *Anthriscus*, *Chaerophyllum*, and *Osmorhiza*, although section *Cacosciadium* and the *C. hirsutum* group each unite species that are so morphologically similar that each is sometimes treated as a single polymorphic species. Members of the *C. hirsutum* group are characterized by ciliate petals (character no. 24); however, single hairs at petal margins also occur in *C. crinitum* and are characteristic for *Osmorhiza* and some members of the *Athamanta* group. The *C. temulum* group includes monocarpic species (1, not shown on the tree) with a predominantly lateral position of umbels (16, not shown); however, these character states are highly homoplastic and are also characteristic for *Myrrhoides nodosa*. There is no single character state that separates the *C. aureum* group from the other members of *Chaerophyllum*.

Discussion

The utility of morphological characters for phylogenetic inference

Our study confirms that morphology is of limited value for estimating phylogenetic relationships in Apioideae, at least at higher taxonomic levels. Such a conclusion is not surprising given the common dissatisfaction among botanists with diagnostic characters used in the taxonomy of umbellifers (e.g., Heywood, 1971, 1982). Katz-Downie et al. (1999) showed that cotyledon shape, pollen morphology, and stomata types that had been evoked to support the classification system of Cerceau-Larrival (1962, 1963, 1965; also Guyot, 1971; Guyot et al., 1980) do not separate major lineages in Apioideae identified using molecular data. Our analyses indicate that those commonly used morphological markers related to habit, life history strategy, and reproductive traits are also highly homoplastic. These characters are all subject to strong selective pressures; hence different lineages may have reached similar adaptive peaks. Bell (1971) pointed out that the inflorescence architecture and floral morphology of umbellifers, generally considered unspecialized, may in fact constitute adaptations to a specific set of pollinators, while Webb (1981) discussed the adaptive significance of andromonoecy and protandry. Jury (1986) underlined similarities in habit, inflorescence, and floral traits of annual members of Caucalideae (i.e., subtribes Torilidinae and Daucinae, in part). Another example of the close connection between morphology and ecology is the diversification of *Anthriscus* (Spalik, 1996, 1997), which fits to Grime's (1988) model of three primary ecological factors, i.e., stress, competition, and disturbance, with the respective strategies being stress resistant, competitive, and ruderal. Different fruit outgrowths such as wings, spines, hairs, etc., are usually explained by different strategies of fruit dispersal. For example, it has been suggested that in heterocarpic members

←

tribal and subtribal divisions identified by Downie et al. (2000a, b). Subclades identified in Scandicinae are also bracketed and are further described in the text. Names of informal groups are enclosed with double quotes to distinguish them from formally recognized taxa.

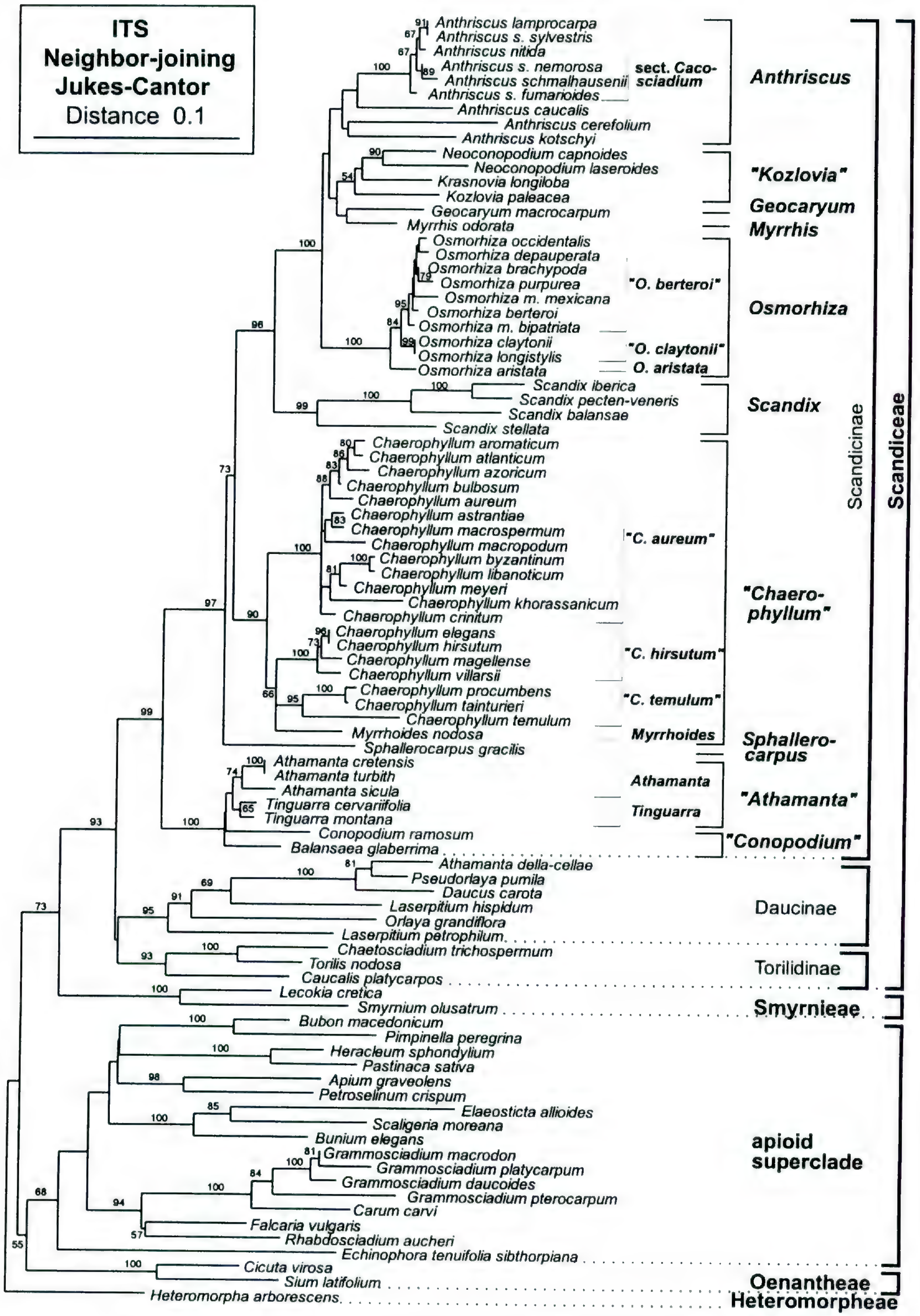


Figure 2. Neighbor-joining tree inferred from 89 nuclear rDNA ITS sequences from Scandiceae subtribe Scandicinae and outgroups. Branch lengths are proportional to distances estimated from the Jukes-Cantor method (note scale bar). Numbers denote bootstrap values for particular nodes; only those > 50% are indicated. Complete taxon names are provided in Table 3. All bracketed groups correspond to Figure 1.

of subtribes Torilidinae and Daucinae, spiny fruits are adapted for long-distance dispersal while naked ones are to maintain the local population (Jury, 1986). Similar heterocarpy has been independently acquired in *Scandix australis* and *S. turgida* in which not only the central fruit may be different from the peripheral ones but the mericarps are heteromorphic, the inner being smooth while the outer is bristled (Hedge & Lamond, 1972c; K. Spalik, pers. obs.).

The traditional emphasis on fruit characteristics for delimiting taxa in Apiaceae has found some confirmation from our analyses. Of the 44 characters examined, 6 from fruit morphology are most congruent with the ITS-derived phylogenies. These include such long-used characters as shape of the primary ribs and the presence of secondary ribs. This result was anticipated as our previous molecular analyses confirmed that most genera of Scandicinae are monophyletic (Downie et al., 2000a), thus suggesting that they were correctly identified as natural groups on the basis of fruit morphology. Other characters have generally appeared unreliable for delimiting taxa. The majority of infrageneric divisions traditionally recognized in *Chaerophyllum* and *Osmorhiza*, erected on the basis of life history, inflorescence architecture, floral morphology, and vegetative characters, are not supported, while newly identified divisions inferred from molecular data find little confirmation from morphology.

The results of the combined analysis, however, show that morphology may be useful at lower taxonomic levels. Adding morphological characters to the molecular data did not substantially change the topology of resulting trees but increased the resolution and bootstrap support for those terminal nodes where molecular data alone were inconclusive (such as within the *Athamanta* and *Conopodium* clades).

Since members of Scandicinae do not share any apomorphic characters, it is surprising that the affinity of its included taxa has been recognized, given that most early botanists were followers of Aristotle's (and Linnaeus's) downward classification by logical division using a limited number of characters rather than Adanson's multi-character upward grouping based on similarity (Mayr, 1982). Their attitude is exemplified by a perfect congruence between their classifications and keys. However, a closer examination of the description and the content of subtribe Scandicinae in different accounts reveals that the rule of logical division was not consistently applied. For instance, Drude (1898) defined Scandiceae based on the presence of calcium oxalate crystals in the aerenchymatic tissue surrounding the carpophore. He placed in subtribe Scandicinae those members of the tribe having oblong fruits with no secondary ridges. However, several species correctly assigned to Scandicinae by Drude, such as some members of *Chaerophyllum*, actually lack these crystals (Calestani, 1905; Koso-Poljansky, 1916). Drude's system, therefore, was probably intuitive and based on multiple characters rather than on single diagnostic characters. It seems that Calestani (1905) tried to follow strictly the rule of logical division, for he separated those species of *Chaerophyllum* that did not have calcium oxalate crystals in the commissure. However, his classification was not much better than that of Drude, and in the case of subtribe Scandicinae much inferior.

Character coding may be another factor leading to poor resolution. Morphological characters can be quite variable, and the typology developed for one taxon is often ineffective for another. When attempting a broader analysis and adding outgroup taxa one has to either mark many characters as inapplicable or exclude them altogether, thus losing resolution at lower taxonomic levels. It is often difficult to establish homologies, hence one gets either a few dubious but potentially more phylogenetically informative states or many unequivocal but often autapomorphic and phylogenetically useless states. For instance, Reduron (1982) developed a detailed typology of petals of umbellifers, but in fact these types were selected from a continuous spectrum of shapes and sizes. Moreover, in species with zygomorphic flowers, several types may occur on a single plant. Similar difficulties arise with fruit and leaf shapes. When adapting such typologies for phylogenetic analysis, there is a danger of "wishful thinking," i.e., unintentional perceiving and consecutive coding character states in such a way as to confirm one's ideas on phylogenetic affinities between taxa. Given the subtle differences seen between some character states in our analysis we may have succumbed to this delusion too. For the morphological analysis of Scandicinae, our departure point was a study of *Anthriscus* (Spalik, 1996, 1997), and this inevitably influenced the choice and coding of characters thereafter.

DEFINITION OF SCANDICINAE

Delimitation of tribes and subtribes on the basis of morphological synapomorphies would significantly speed up the reclassification of umbellifers by allowing preliminary sorting of taxa prior to more costly molecular investigations. However, our anal-

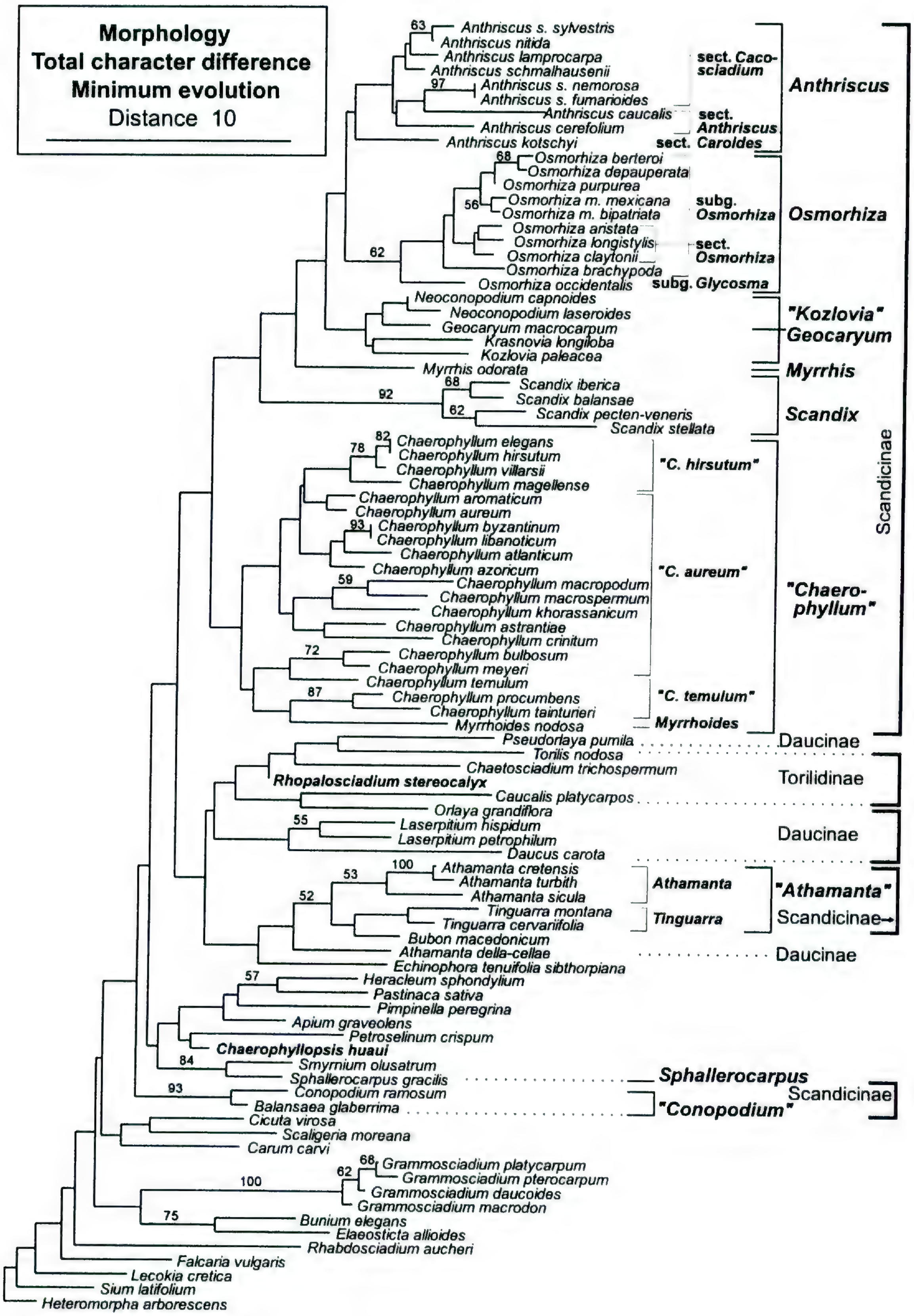


Figure 3. Minimum evolution tree inferred from 44 morphological characters for 91 representatives of Scandiceae subtribe Scandicinae and outgroups. Branch lengths are proportional to distances estimated using total character difference (total branch length = 355.92; note scale bar). Bootstrap values > 50% are indicated along the nodes. Boldface

yses suggest that such an aid for the umbellifer taxonomist is unlikely. None of the morphological characters that we have examined is synapomorphic for Scandicinae, although the taxa included are generally similar and share many common features. Scandicinae may be defined generally by possessing ovate to lanceolate and ciliate bracteoles, and narrowly ovate to linear oblong, laterally compressed, beaked fruits lacking secondary ridges. Several members depart from this definition: for instance *Anthriscus kotschyi* has non-ciliate bracteoles while many species of *Chaerophyllum* do not have a pronounced beak. Additional characters include reduced sepals and the absence of oil ducts in the petals; these characters, however, are not found in basal Scandicinae, i.e., the *Athamanta* and *Conopodium* groups. An aculeate, areolate, and shiny fruit epidermis and angular primary ribs adequately demarcate "crown" Scandicinae (*Anthriscus, Geocaryum, Kozlovia, Krasnovia, Myrrhis, Neoconopodium,* and *Osmorhiza*) from all other umbellifers. *Scandix* may be identified as a close relative to this clade due to its aculeate and areolate fruit epidermis. It seems, therefore, that among potential members of Scandicinae, it would be easy to identify those that belong to the "crown" clade. However, those related to *Sphallerocarpus, Conopodium,* or *Athamanta* may not readily be recognized as the members of the subtribe on the basis of morphology alone.

PHYLOGENETIC AFFINITIES OF *RHOPALOSCIADIUM* AND *CHAEROPHYLLOPSIS*

The monotypic genus *Rhopalosciadium* was described from Iran based on a single gathering and originally placed in Scandicinae, presumably on account of its linear fruits (Rechinger, 1952). Later, based on its fruit indumentum, the genus was transferred to tribe Caucalideae. Its closest relative was thought to be *Torilis,* with which the plant was growing in the wild (Hedge & Lamond, 1980; Rechinger, 1987b). In our combined analysis (Fig. 4), *Rhopalosciadium* was grouped with *Chaetosciadium* in Torilidinae. The type material of *Rhopalosciadium stereocalyx* at W, the data source for this analysis, has only immature fruits with obsolete primary and secondary ridges. These ridges are also inconspicuous in *Chaetosciadium,* a monotypic genus apparently nested within *Torilis* (Lee & Downie, 1999). Bristles covering the ovary of *Rhopalosciadium,* as seen under high magnification of a dissecting microscope, are indeed similar to those of *Torilis,* a member of Torilidinae sensu Downie et al. (2000a), but different from those in Scandicinae. *Rhopalosciadium* should therefore be recognized in subtribe Torilidinae. Given that the only collection of this taxon was found growing with *Torilis,* it may just be an aberrant form of the latter.

Chaerophyllopsis huaui is endemic to China. Herbarium material of this species is very rare in botanical collections, and we have seen only one specimen (Yunnan, 26 July 1906, *Ducloux 4565,* P) but this lacked mature fruits. Based on the analysis of this incomplete specimen, this taxon should be excluded from Scandiceae and transferred to the apioid superclade. Indeed, Pimenov and Leonov (1993) following Sheh and Su (1987) treated it in subtribe Apieae.

THE TAXONOMIC POSITION OF MONOTYPIC GENERA

There is general agreement that all taxonomic categories, with the exception of species, are arbitrary and apart from monophyly there are no universal criteria by which they should be distinguished. The Linnaean hierarchy of names has been a tool for species recognition in which genera play an important role. The binomial not only denotes a particular species but also indicates its closest relatives (i.e., congeners). As an aid to species identification, monotypic genera are therefore useless and taxonomists should avoid creating them unless they indeed represent isolated lineages significantly distinct from their sister taxa. Of the 455 genera of Umbelliferae listed by Pimenov and Leonov (1993), 41% are monotypic and 26% comprise only 2 or 3 species each. Similarly, of the 16 genera constituting Scandiceae subtribe Scandicinae, 7 (44%) are monotypic (Table 1). Based on our combined study, the number of monotypic genera in Scandicinae may satisfactorily be reduced, as only *Sphallerocarpus* and *Myrrhis* represent isolated evolutionary lineages.

Contrary to the molecular analyses, which allied *Myrrhis odorata* with *Geocaryum* (Figs. 1, 2), the combined analysis placed *M. odorata* sister to *Osmorhiza* (Fig. 4) in accordance with the relationship proposed by Lowry and Jones (1984). *Sphallerocarpus gracilis* occurs in the Far East, in contrast to

←

marks *Chaerophyllopsis* and *Rhopalosciadium,* which were not available for ITS study. Bracketed names are similar to those provided in Figures 1 and 2; additional detail is provided within the *Anthriscus* and *Osmorhiza* clades.

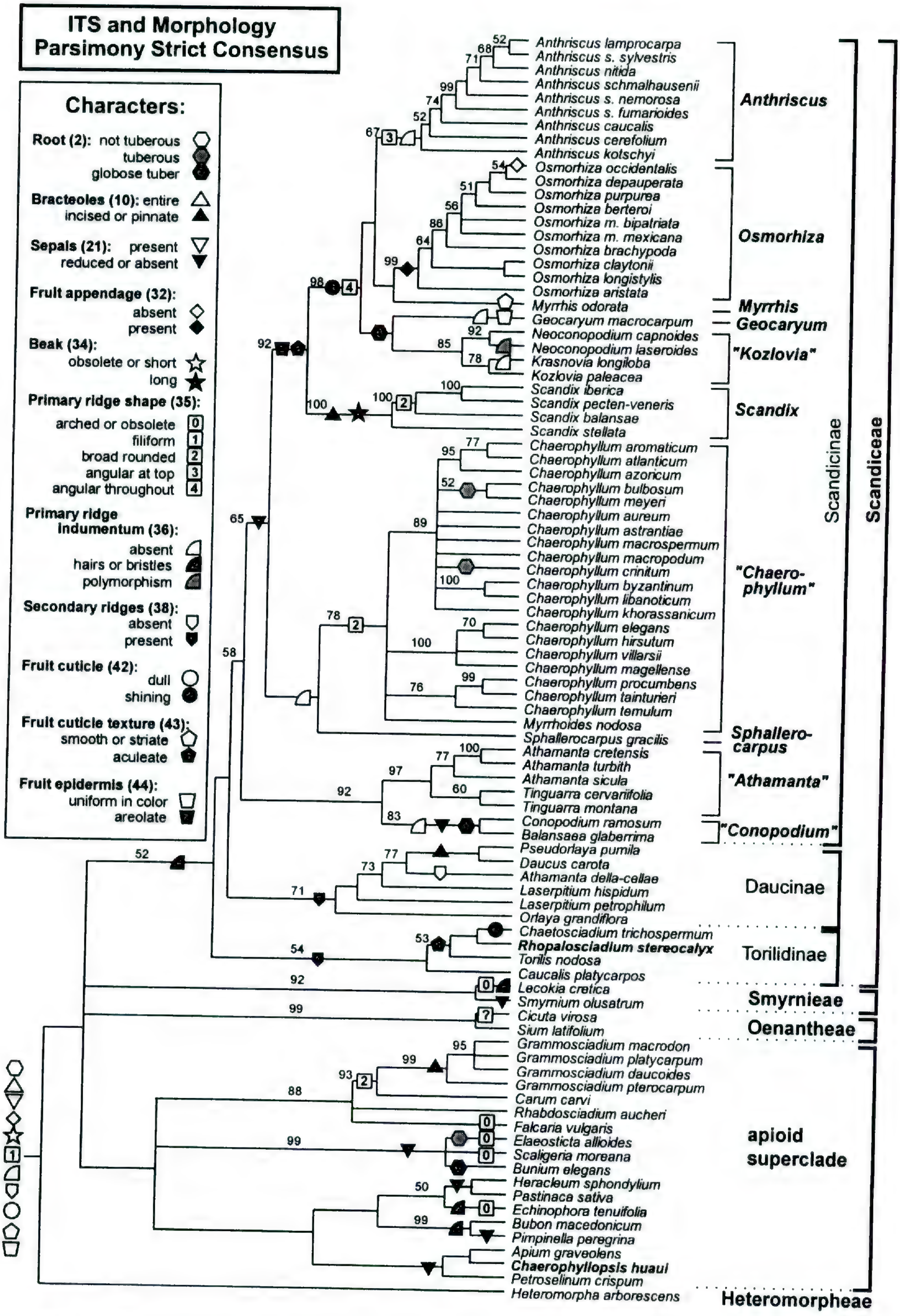


Figure 4. Strict consensus of 544 minimal length 2272-step trees inferred from equally weighted maximum parsimony analysis of combined morphological and ITS sequence data for 91 representatives of Scandiceae subtribe Scandicinae and outgroups (CI = 0.321 excluding uninformative characters, RI = 0.717). Boldface indicates *Chaerophyl-*

Table 5. Number of steps (NS) and consistency (CI), retention (RI), and rescaled consistency (RC) indices of 44 morphological characters estimated by mapping these characters on a Jukes-Cantor/neighbor-joining tree inferred from ITS sequences. Characters are listed according to descending RC values; character numbers refer to those presented in Appendices 1 and 2.

Character	NS	CI	RI	RC
42. Cuticle appearance	2	0.50	0.96	0.48
44. Epidermis coloration	2	0.50	0.96	0.48
32. Fruit appendage	2	0.50	0.88	0.44
38. Secondary ridges	2	0.50	0.86	0.43
35. Primary ridge shape	10	0.40	0.89	0.36
43. Cuticle texture	3	0.33	0.93	0.31
15. Umbel width	2	0.50	0.50	0.25
21. Sepals	7	0.29	0.82	0.23
41. Fruit indumentum	24	0.38	0.58	0.22
10. Bracteole division	7	0.29	0.64	0.18
36. Primary ridge indumentum	9	0.22	0.79	0.18
4. Lobe orientation	8	0.25	0.63	0.16
24. Petal margin	9	0.22	0.71	0.16
37. Mericarp compression	7	0.29	0.50	0.14
8. Bract margin	4	0.25	0.50	0.13
33. Commissure	9	0.11	0.81	0.09
19. Central flower	9	0.11	0.72	0.08
22. Sepal indumentum	6	0.17	0.50	0.08
2. Root	9	0.22	0.30	0.07
26. Oil ducts in petals	9	0.11	0.62	0.07
28. Crown of hairs at fruit base	9	0.11	0.62	0.07
29. Pedicel indumentum	14	0.14	0.48	0.07
31. Fruit shape	25	0.12	0.60	0.07
34. Beak	17	0.12	0.59	0.07
23. Petal incision	12	0.08	0.68	0.06
5. Shape of basal leaf lobes	10	0.10	0.47	0.05
6. Shape of cauline leaf lobes	16	0.13	0.42	0.05
11. Bracteole margin	22	0.09	0.57	0.05
13. Bracteole shape	10	0.10	0.53	0.05
14. No. of umbellets in primary umbel	12	0.08	0.66	0.05
16. Umbel position	15	0.13	0.41	0.05
3. Leaf division	38	0.11	0.37	0.04
7. Bracts	25	0.08	0.44	0.04
20. Outer flowers	15	0.07	0.67	0.04
30. Pedicels of fruit	8	0.13	0.30	0.04
9. No. of bracteoles	35	0.09	0.33	0.03
12. Bracteole indumentum	17	0.06	0.45	0.03
18. Disc male flowers	9	0.11	0.27	0.03
25. Petal indumentum	11	0.09	0.38	0.03
27. Stylopodium shape	29	0.07	0.44	0.03
1. Reproductive strategy	41	0.05	0.38	0.02
17. Peripheral flowers	2	0.50	0	0
39. Secondary ridge appendages	3	0.67	0	0
40. Tubercles at fruit surface	12	0.08	0	0

←

lopsis and *Rhopalosciadium* for which ITS data were not available. Numbers along nodes denote bootstrap values; only those > 50% are indicated. The morphological characters that are most useful for delimiting genera and suprageneric lineages are indicated. Character numbers refer to those presented in Appendices 1 and 2.

other Scandicinae, which have their center of distribution in the Mediterranean region. This East Asiatic lineage may actually be represented by more than one species, with one possible candidate being *Vicatia*, which was included in *Sphallerocarpus* by Koso-Poljansky (1916). In contrast, both molecular (Fig. 2) and morphological (Fig. 3) analyses indicate that *Myrrhoides* is nested within *Chaerophyllum*, although in some trees (not shown) these genera constitute sister taxa. We therefore see no justification for separating these two genera. Such a treatment is not novel. Linnaean *Scandix nodosa* L., the basionym of *Myrrhoides nodosa* (Dandy & Cannon, 1968), was earlier transferred to *Chaerophyllum* by Crantz (1767) and recognized there by de Candolle (1829, 1830). The correct name of this species in *Chaerophyllum* is therefore *C. nodosum* (L.) Crantz.

The relationships among basal Scandicinae are obscure. Phylogenetic analysis of combined data supports the monophyly of each of the *Athamanta* and *Conopodium* groups (Fig. 4), contrary to separate analysis of ITS data (Fig. 2), where the latter is clearly not monophyletic. Based on morphology, the inclusion of *Tinguarra* into *Athamanta* should also be considered but this transfer needs confirmation from fruit anatomy. *Balansaea* was included in *Conopodium* by Engstrand (1973), and although this finds support from morphology (Fig. 3), the monophyly of *Conopodium* is not confirmed by our ITS analyses.

The *Kozlovia* clade encompasses four species (*K. paleacea, Krasnovia longiloba, Neoconopodium capnoides,* and *N. laseroides*) that are similar in habit and closely related based on molecular data. Their geographic distribution also supports their close relationship. *Kozlovia paleacea* and *Krasnovia longiloba* are Central Asiatic, with *Krasnovia* extending northeast to China and *Kozlovia* reaching Afghanistan (Korovin, 1950; Schischkin, 1950c; Rechinger, 1987a). *Neoconopodium* has a Himalayan distribution; *N. laseroides* is western and occurs in Afghanistan and Pakistan, while *N. capnoides* is eastern, occurring in Pakistan, Kashmir, and the Indian Himalayas (Hedge & Lamond, 1980, 1987; Pimenov & Kljuykov, 1987). The combined analysis (Fig. 4) indicates that the East Mediterranean *Geocaryum* (which is also a geophyte) is sister to this group. The morphological variation observed within the *Kozlovia* clade is comparable to that exhibited by *Anthriscus* or *Chaerophyllum* and, consequently, a single genus should be recognized.

Kozlovia Lipsky, Trudy Imp. S.-Peterburgsk. Bot. Sada 23: 146. 1904. TYPE: *Kozlovia paleacea* (Regel & Schmalh.) Lipsky (basionym: *Albertia paleacea* Regel & Schmalh.).

Albertia Regel & Schmalh., Trudy Imp. S.-Peterburgsk. Bot. Sada 5: 603. 1878. Non W. P. Schimper 1837. TYPE: *Albertia paleacea* Regel & Schmalh.

Krasnovia Popov ex Schischk., Flora SSSR 16: 591. 1950. TYPE: *Krasnovia longiloba* (Kar. & Kir.) Popov ex Schischk. (basionym: *Sphallerocarpus longilobus* Kar. & Kir.).

Neoconopodium Pimenov & Kljuykov, Feddes Repert. 98: 377. 1987. TYPE: *Neoconopodium capnoides* (Decne.) Pimenov & Kljuykov (basionym: *Butinia capnoides* Decne.).

1. **Kozlovia paleacea** (Regel & Schmalh.) Lipsky, Trudy Imp. S.-Peterburgsk. Bot. Sada 23: 146. 1904. *Albertia paleacea* Regel & Schmalh., Trudy Imp. S.-Peterburgsk. Bot. Sada 5: 606. 1878. TYPE: Uzbekistan. Between Karasu and Katty Kurgan, 26 Apr. 1869, *O. Fedchenko* (lectotype, designated by Vinogradova (1999), LE not seen).

Even though we have not seen the *Fedchenko* type specimen, we rely on the expert confirmation of its status by V. M. Vinogradova. This lectotype for *Albertia paleacea* was chosen by her among her discussion of Apiaceae types at the Komarov Botanical Institute (Vinogradova, 1999) and later also confirmed to us (Vinogradova, pers. comm.). In the revision of *K. paleacea* for the *Flora Iranica*, Rechinger (1987a) indicated A. Regel as the collector of the type specimen; he did not, however, examine any type collections.

Kozlovia paleacea differs from the other members of this genus in having leaves with broad and deeply lobed ultimate segments, ciliate bracteoles, and ovate fruits with primary ridges covered with prominent hyaline bristles.

2. **Kozlovia capnoides** (Decne.) Spalik & S. R. Downie, comb. nov. Basionym: *Butinia capnoides* Decne., in Jacquem., Voy. Bot. 70: tab. 80. 1844. *Chaerophyllum capnoides* (Decne.) Benth., in Benth. & Hook. f., Gen. Pl. 1: 898. 1867. *Conopodium capnoides* (Decne.) Koso-Pol., Bull. Soc. Imp. Naturalistes Moscou 29: 206. 1916. *Neoconopodium capnoides* (Decne.) Pimenov & Kljuykov, Feddes Repert. 98: 377. 1987. SYNTYPES: India. (Punjab?): between Gereti and Gougoulgaon, 2400 m; Kashmir: Ouri and Nouchaira, banks of the Djhelone, 1980 m, *Jacquemont 308* (K not seen; P not seen), *920* (P not seen).

In their discussion on Asiatic Scandiceae, Hedge and Lamond (1980) revised this species, placing it in *Chaerophyllum*. Both in that revision and in a later treatment for the *Flora Iranica* (Hedge & La-

mond, 1987), they assigned both *Jacquemont* syntypes to *K. capnoides*.

Kozlovia capnoides differs from the other species in having leaves with entire, oblong ultimate segments and oblong-cylindrical glabrous fruits with occasional delicate bristles along primary ridges.

3. **Kozlovia laseroides** (Hedge & Lamond) Spalik & S. R. Downie, comb. nov. Basionym: *Chaerophyllum laseroides* Hedge & Lamond, Notes Roy. Bot. Gard. Edinburgh 38: 252. 1980. *Chaerophyllum aquilegifolium* Rech. f. & Riedl, Dan. Biol. Skr. 13, 4: 44. 1963, nom. illeg., non Koso-Pol. 1916. *Neoconopodium laseroides* (Hedge & Lamond) Pimenov & Kljuykov, Feddes Repert. 98: 378. 1987. TYPE: Afghanistan. Nuristan: Pech (Parun) Valley between Chetras and Wama, *Kerstan 851* (holotype, W not seen; isotype, HAL not seen).

The *Kerstan* holotype was verified by Hedge and Lamond (1980, 1987) for the revision of this species for the *Flora Iranica*.

Kozlovia laseroides differs from its congeners in possessing leaves with ovate and petiolulate ultimate segments and oblong-cylindrical fruits covered with antrorse bristles.

4. **Kozlovia longiloba** (Kar. & Kir.) Spalik & S. R. Downie, comb. nov. Basionym: *Sphallerocarpus longilobus* Kar. & Kir., Bull. Soc. Imp. Naturalistes Moscou 14: 432. 1841. TYPE: Kazakhstan. Tarbagatai Mts., by Dschanybek river, 1840, *Karelin & Kirilov s.n.* (LE not seen, checked by V. M. Vinogradova, pers. comm.).

The identity of the type of this species was confirmed to us by V. M. Vinogradova.

Kozlovia longiloba differs from the other species of this genus in having leaves with linear lobes and ovate, tuberculate fruits.

ANTHRISCUS

Our results show that neither morphology nor ITS data fully support the present classification of *Anthriscus*, as section *Anthriscus* is not monophyletic. Phylogenetic analyses of a data set comprising *Anthriscus* only, based on a larger set of morphological characters than that used here, confirmed the monophyly of each of its three sections (Spalik, 1996). However, the character states that identify these sections, although unique when only *Anthriscus* is considered, are quite common among other umbellifers (Spalik, 1997). For instance, characters that support the monophyly of section *Anthriscus*, such as weak protandry, short and straight styles, and an annual life history strategy, are also typical for other annual monocarpic umbellifers (Jury, 1986), and may be interpreted as adaptations to disturbed habitats (Spalik, 1996). Therefore, section *Anthriscus* is likely polyphyletic, and *A. cerefolium* should be placed in a monotypic section *Cerefolium* (Fabr.) Neilr. (Neilreich, 1859). Based on analysis of partial ITS data, a third member of section *Anthriscus*, *A. tenerrima*, is sister to *A. caucalis* and should therefore be retained in this section (K. Spalik & S. Downie, unpublished data).

Although the monophyly of section *Cacosciadium* has been confirmed (Figs. 1, 2), the affinities among its members are not unambiguously resolved. This section comprises four species (*A. sylvestris*, *A. nitida*, *A. lamprocarpa*, and *A. schmalhausenii*) and all were included in the present study. *Anthriscus sylvestris* is further divided into four subspecies (Reduron & Spalik, 1995; Spalik, 1996, 1997) but two of these, subsp. *nemorosa* and subsp. *fumarioides*, have been previously recognized as good species (Cannon, 1968). In contrast, the specific status of *A. nitida* and *A. lamprocarpa* has been questioned (Thellung, 1926; Hedge & Lamond, 1972b). Spalik (1996) suggested that *A. sylvestris* is paraphyletic with respect to *A. nitida*, *A. lamprocarpa*, and *A. schmalhausenii*, the latter three species likely evolving through speciation of isolated peripheral populations of the former. These three species seem to be well separated from the *A. sylvestris* complex both by qualitative and quantitative characters, while there is no morphological hiatus between other taxa from this group; consequently, the latter are recognized as subspecies or varieties of *A. sylvestris*. Our previous ITS study also encompassed Asiatic and East African representatives of *A. sylvestris*; these were so similar morphologically to the European plants that they all were included in subspecies *sylvestris* (Downie et al., 2000a). However, these ITS data did not confirm this affinity. Instead, a close relationship was suggested among the European smooth-fruited taxa (i.e., *A. sylvestris* subsp. *sylvestris*, *A. sylvestris* subsp. *alpina*, and *A. nitida*), *A. lamprocarpa*, and a representative of *A. sylvestris* from northeastern Asia. Another clade comprised the East African and remaining Asiatic members of the *A. sylvestris* complex, suggesting a primary split of the distribution into northwestern and southeastern populations.

The close affinity between Levantine *A. lamprocarpa*, represented in our molecular study by one accession from Jordan, and the European popula-

tions of *A. sylvestris* is not readily explained, as one would expect the former to be related to either parapatric *A. sylvestris* subsp. *nemorosa* from Turkey or East African representatives of subspecies *sylvestris*. It has been suggested that *A. lamprocarpa* originated from an isolated population of *A. sylvestris* subsp. *sylvestris* that may have reached the Middle East from montane localities in North Africa, which themselves may have originated from Iberian Peninsula stock when this area was covered with forests during the Pleistocene (Spalik, 1996). An analysis of North African *A. sylvestris* could address this hypothesis. One may also postulate a more recent origin of this species by the occasional introduction of *A. sylvestris* into the Middle East in antiquity and its subsequent adaptive evolution. *Anthriscus sylvestris* is a competitive-ruderal species (Grime et al., 1988) that has been recently introduced into North America; given the extensive trade exchange in the Mediterranean region, such a scenario is quite possible.

This study does not answer the question as to how many species there are within the *A. sylvestris* complex. Morphological differences suggest that there are reproductive barriers among *A. nitida*, *A. lamprocarpa*, *A. schmalhausenii*, and *A. sylvestris* (Spalik, 1996, 1997). However, other taxa included in the latter, particularly montane populations from Africa and Asia, may also be reproductively isolated and may deserve specific status despite their lack of good diagnostic features. In a biosystematic study of *Geocaryum*, Engstrand (1977) demonstrated by means of hybridization experiments that many populations hitherto regarded as conspecific are partly or entirely reproductively isolated. Consequently, instead of 3 species (Ball, 1968), he recognized as many as 15. However, these taxa are practically indistinguishable based on morphology. *Anthriscus sylvestris* may well represent another such complex of cryptic species with different degrees of reproductive isolation. Contrary to many groups of angiosperms, interspecific hybridization is thought to be exceedingly rare in umbellifers (Bell, 1971). The example of *Geocaryum* suggests, however, that Apiaceae may not be exceptional. Due to their obscure morphology many such cases may simply escape the attention of taxonomists.

CHAEROPHYLLUM

Contrary to *Anthriscus* and *Osmorhiza*, *Chaerophyllum* has not been revised recently. It is the most diversified genus in the subtribe and includes over 30 species; hence an infrageneric classification would be advantageous. The only modern account containing infrageneric divisions is that of Schischkin (1950b), being mostly based on the work of Koso-Poljansky (1916, 1920, 1923). The names in these treatments, however, are problematic. Koso-Poljansky (1916) did not explicitly indicate nomenclatural types for his subgenera nor did he provide species lists, while the names of series introduced by Schischkin (1950b) were not validly published as he did not provide Latin diagnoses. Additionally, this treatment encompassed only those taxa of the former Soviet Union, and hence some European, Asiatic, and American members of this genus were not considered (Table 2).

Schischkin (1950b) generally based his classification on plant habit, but the content of his divisions occasionally did not coincide with the indicated diagnostic characters. For instance, although his subgenus *Buniomorpha* was defined as encompassing plants with tuberous roots it also included *C. temuloides*, a taxon now included in *C. aureum*, which does not have such a root (Hedge & Lamond, 1972a). His subgenus *Golenkinianthe* was defined by members possessing fruits borne only by central flowers, a feature that is also found in *Grammosciadium* and *Echinophora*. However, central perfect flowers occur practically in all species of *Chaerophyllum*, while in *C. macrospermum*, the only species placed by Schischkin in subgenus *Golenkinianthe*, fruits may also be borne by outer perfect flowers, particularly those of the primary umbels (K. Spalik, pers. obs.). The classification of Schischkin (1950b) has not been confirmed by this study. Instead, both ITS data alone (Figs. 1, 2) and combined molecular and morphological data (Fig. 4) indicate that there are four distinct lineages. We refer to these as (1) the *C. temulum* group, (2) the *C. hirsutum* group, (3) the *C. aureum* group, and (4) *C. nodosum* (≡ *Myrrhoides nodosa*). These groups are not morphologically distinct enough to justify their generic status, so we recognize them as sections of *Chaerophyllum*. Our treatment is incomplete, as not all currently recognized species were available for molecular study. Therefore, we do not attempt a detailed revisionary study of this genus but rather provide a framework for such studies in the future.

Chaerophyllum L., Sp. Pl. 258. 1753. TYPE: *Chaerophyllum temulum* L., Sp. Pl. 258. 1753, *Herb. Linn. 365.3* (lectotype, designated by Reduron & Jarvis (1992), LINN not seen).

Section 1. Chaerophyllum sect. **Chaerophyllum**

Annuals or biennials. Umbels mostly lateral or both lateral and terminal. Male flowers absent or rare. Petals glabrous, non-ciliate. Fruits ovate, glabrous, rarely pilose.

Species included: *C. procumbens, C. tainturieri, C. temulum.*

This section encompasses monocarpic species distributed in Europe (*C. temulum*) and in North America (*C. procumbens, C. tainturieri*). Such a disjunction is generally rare; most groups of vicarious species that occur both in Europe and North America are also found in the Far East (Meusel et al., 1978), supporting the hypothesis that these species diverged from a once widespread common ancestor with circumboreal distribution. The absence of representatives of this clade from Asia suggests that the North American species originated from an incidental dispersion of seeds from European stock, probably by vagrant birds. American species differ from their European cousin in the absence of male flowers and the almost completely reduced corollas, i.e., characters that are typical for self-pollinating species.

Section 2. Chaerophyllum sect. **Dasypetalon** Neilr., Fl. Nied.-Oesterr. 645. 1859. TYPE: *Chaerophyllum hirsutum* L., Sp. Pl. 258. 1753.

Chaerophyllum sect. *Rhynchostylis* (Tausch) Calest., Webbia 1: 188. 1905. *Rhynchostylis* Tausch, Flora 17: 343. 1834. Non Blume 1825. TYPE: *Rhynchostylis hirsutus* Tausch (*Chaerophyllum hirsutum*).

Perennials. Umbels mostly terminal. Male flowers frequent. Petals glabrous, ciliate. Fruits oblong-ovate to oblong.

Species included: *C. hirsutum, C. elegans, C. magellense, C. villarsii.*

The members of this section are morphologically similar, prompting some authors (e.g., Thellung, 1926) to regard these taxa as conspecific. The differences are indeed small and include mostly leaf characters. All taxa are perennials and are characterized by distinctly ciliate petals. The importance of this character was stressed by Neilreich (1859) who divided Austrian species of *Chaerophyllum* into two sections, *Leiopetalon*, with glabrous petals, and *Dasypetalon*, with hairy petals. The first section comprised the type of the genus, and therefore it is synonymous with section *Chaerophyllum*. Neilreich (1859) included two species in section *Dasypetalon*, *C. hirsutum* and *C. villarsii*, but he did not specify its nomenclatural type. Both species fully conform to the diagnosis of the section. *Chaerophyllum hirsutum* is an older name and has priority when these taxa are treated as conspecific, and therefore it seems to be a better choice.

Chaerophyllum hirsutum has not yet been lectotypified. Of the extant Linnaean specimens of this species, those at LINN and S do not seem to be connected with the entry in the first edition of Linnaeus's (1753) *Species plantarum*. The original material available for lectotypification includes, therefore, two BM specimens (*Herb. Clifford*, 101: *Chaerophyllum 2* and *2*β) and figure 6 (plate 10) from Morison (1699) cited in the protologue (C. E. Jarvis, pers. comm.). Linnaeus (1753) cited also a plate from Haller's (1745) edition of Ruppius's *Flora jenensis*. However, in the second edition of the *Species plantarum* (Linnaeus, 1762), this plate is referred to *C. aureum*. Since we have not had the possibility to examine the original material, we refrain from lectotypification of *C. hirsutum*.

Section 3. Chaerophyllum sect. **Physocaulis** DC., Coll. Mém. 5: 59. 1829. TYPE: *Chaerophyllum nodosum* (L.) Crantz, Class. Umbell. Emend. 76. 1767. Basionym: *Scandix nodosa* L., Sp. Pl. 257. 1753; *Herb. Linn.* 364.6 (lectotype, designated by Hedge & Lamond (1987), LINN not seen).

Densely setose annuals. Umbels mostly lateral. Male flowers scarce. Petals setose, non-ciliate. Fruits ovate, setose.

Chaerophyllum nodosum, the only member of this section, differs from other species of *Chaerophyllum* in having setose fruits with very broad primary ridges. These ridges nearly touch each other, and hence valleculae are inconspicuous and colored similarly to the ridges; in contrast, these regions are often of different color in other species. However, fruit indumentum and broad ridges are not unique to *C. nodosum*. The members of section *Chaerophyllum* may have pubescent fruits (although not setose), while the width of the ridges (as determined by the width of the vascular bundles) is quite variable in the genus (K. Spalik, A. Wojewódzka & S. Downie, unpublished data).

This monotypic section was first recognized by de Candolle (1829, 1830) and raised to generic level by Tausch (1834). Later, the generic name *Physocaulis* (DC.) Tausch was rejected by Dandy and Cannon (1968) in favor of the forgotten but earlier *Myrrhoides*. The ITS and combined analyses place *Chaerophyllum nodosum* either as a sister to remaining members of the genus or nested within it, supporting the treatment of de Candolle (1829).

Section 4. Chaerophyllum sect. **Chrysocarpum** Spalik & S. R. Downie, sect. nov. TYPE: *Chaerophyllum aureum* L., Sp. Pl. ed. 2. 370. 1762, *Herb. Linn. 365.8* (original material, LINN not seen), plate 5 in Haller (1745), (C. E. Jarvis, pers. comm.).

A sectionibus *Chaerophyllo* et *Physocaule* radice perenni vel bienni, a sectione *Leiopetalone* petalis non ciliatis differt.

Biennials or perennials. Umbels mosly terminal or both terminal and lateral. Male flowers usually present. Petals non-ciliate, usually glabrous, rarely hairy. Fruits oblong-ovate to cylindrical, glabrous, rarely pilose.

Etymology. From *chryso-*, golden, and *carpos*, fruit, since many species in this section (including the type species) are characterized by straw-yellow fruits.

Species included: *C. aromaticum*, *C. astrantiae*, *C. atlanticum*, *C. aureum*, *C. azoricum*, *C. bulbosum*, *C. byzantinum*, *C. crinitum*, *C. hakkiaricum*, *C. khorassanicum*, *C. libanoticum*, *C. macropodum*, *C. macrospermum*, *C. meyeri*, *C. nivale*. Two of these, *C. hakkiaricum* and *C. nivale*, were not considered herein but are included based on our prior study (Downie et al., 2000a).

Section *Chrysocarpum* is morphologically and ecologically diversified, and we were not able to find any single morphological feature separating it from any other section of *Chaerophyllum*. Its members differ from representatives of sections *Chaerophyllum* and *Physocaulis* in their predominantly perennial habit. Some species are, however, biennials and these may have tuberous roots (*C. bulbosum*, *C. crinitum*). Several species have pubescent petals with hairs occurring also at the margin. Such petals are superficially similar to those ciliate petals characteristic of members of section *Dasypetalon*.

Maximum parsimony analysis (Fig. 1) did not totally resolve relationships within this clade; however, the affinities inferred from ITS data using distance methods are largely congruent with the geographic distribution of these taxa. Neighbor-joining analysis (Fig. 2) suggests a division into four major lineages; however, only one of these is supported by a high bootstrap value (88%). This lineage encompasses species that have a predominantly European distribution (*C. aureum*, *C. bulbosum*, *C. azoricum*, *C. atlanticum*, and *C. aromaticum*), whereas all other species in the section occur in the Middle East.

The type species of this section, *Chaerophyllum aureum*, has not been properly lectotypified. Although Hedge and Lamond (1987) indicated LINN 365.7 as the type, it is probably not an original element for the name, as Linnaeus did not annotate it as belonging to this species; LINN 365.8 may be original material (C. E. Jarvis, pers. comm.); however, this specimen probably does not represent *C. aureum* as this species is currently recognized (J.-P. Reduron, pers. comm.). In the protologue, Linnaeus (1762) cited also plate 5 from Haller (1745) that is identifiable as *C. aureum*. We refrain from lectotypification of this name until we examine the extant herbarium material. In the first edition of the *Species plantarum* (Linnaeus, 1753), some earlier references that are connected with *C. aureum* as this species is now recognized were included either in *C. sylvestre*, the basionym of *Anthriscus sylvestris* (Reduron & Spalik, 1995), or in *C. hirsutum* (e.g., plate 5 in Haller, 1745).

With no molecular data available, 12 currently recognized species remain outside this new classification. These are *C. angelicifolium*, *C. borodinii*, *C. coloratum*, *C. confusum*, *C. creticum*, *C. heldreichii*, *C. humile*, *C. leucolaenum*, *C. reflexum*, *C. roseum*, *C. rubellum*, and *C. villosum*. These are either biennials with tuberous roots or perennials; hence it is unlikely that they belong to either section *Chaerophyllum* or section *Physocaulis*. Neither are they likely related to section *Dasypetalon*, since all have non-ciliate petals. The most likely placement may well be in section *Chrysocarpum*. However, some of these species may comprise additional lineages. For instance, on the basis of anatomical characters, three East Mediterranean species were transferred by Calestani (1905) to *Grammosciadium* sect. *Chrysophae* (*C. creticum*, *C. coloratum*) and section *Heldreichia* (*C. heldreichii*). Based on our preliminary analyses of morphological and anatomical characters of the fruits, such a placement is unlikely (K. Spalik & A. Wojewódzka, unpublished data).

OSMORHIZA

Of all the genera included in Scandicinae, *Osmorhiza* is exceptional in having a predominantly New World distribution. It includes 10 species (with 9 considered herein), of which only *O. aristata* occurs in the Old World (Table 2). *Osmorhiza glabrata* (not considered here) is restricted to the central Andes, while the remaining 8 species are distributed in North and Central America. Western North America is considered the center of origin of the genus (Lowry & Jones, 1984). The taxonomic division developed by Constance and Shan (1948) and later modified by Lowry and Jones (1984) im-

plies that the earliest branch is *O. occidentalis*, which constitutes the monotypic subgenus *Glycosma*. Its distribution includes western North America, thus supporting the American origin of the genus. Subgenus *Osmorhiza* is divided into three sections: *Osmorhiza*, *Mexicanae*, and *Nudae* (Lowry & Jones, 1984; see Table 2). The first encompasses Asiatic *O. aristata* and two closely related species from eastern North America, *O. claytonii* and *O. longistylis*. These three species are so morphologically similar that they are sometimes treated as conspecific (e.g., Gray, 1859; Kuntze, 1891; Boivin, 1968). Lowry and Jones (1979) showed that the two American members of this section are completely separable from each other and that there are no intermediate specimens which would have suggested hybridization.

Three hypotheses have been invoked to explain the similarity between the Asiatic and American members of section *Osmorhiza*. Constance and Shan (1948) suggested that either there had been a relatively recent contact between the Asian and North American populations through Beringia, or these species have differentiated slowly from a once widespread common ancestral population. Li (1972) postulated that these morphological similarities may be the result of parallel evolution due to similar ecological and geographic factors. Lowry and Jones (1984) opted for the second hypothesis arguing that due to more or less stable, mesic conditions in these areas, which constituted the two most important refugia of Tertiary flora in the Northern Hemisphere, the species may have remained relatively unchanged over long periods. The geographic distribution of the members of the remaining sections *Mexicanae* and *Nudae* supports the western North American origin of the genus and its differentiation and dispersion to South America by a step-wise migration through the tropics along a route now marked by members of section *Mexicanae* (Lowry & Jones, 1984). This scenario is also partly supported by the analysis of morphological data that places *O. occidentalis* as sister to the remaining taxa and suggests the monophyly of section *Osmorhiza* (Fig. 3). A different scenario arises from the analyses of ITS (Figs. 1, 2) or combined data (Fig. 4). These results strongly suggest that section *Osmorhiza* is paraphyletic with respect to the other members of the genus as *O. aristata* is sister to all other *Osmorhiza* species. This indicates an Asiatic rather than an American origin of the genus. Therefore, the similarity between *O. aristata*, *O. claytonii*, and *O. longistylis* may be plesiomorphic, which supports the hypothesis of slow morphological change. The eastern North American members of section *Osmorhiza* are a sister group to a clade formed by *O. occidentalis* and representatives of sections *Mexicanae* and *Nudae*. For this clade, southwestern North America seems to be the center of distribution and origin. The results of the ITS (Figs. 1, 2) and combined analysis (Fig. 4), although contradictory to the conclusions inferred from morphology alone (Fig. 3), are no less congruent with the phytogeographic data.

The present subgeneric classification of *Osmorhiza* seems to be untenable. An alternative division of *Osmorhiza* congruent with the phylogenies inferred from molecular and combined data would include three taxa (subgenera or sections) encompassing one, two, and six species respectively (omitting *O. glabrata*, as this species was not available for our molecular studies). Such a division is practically useless as an aid in species recognition. Furthermore, as compared to *Anthriscus* and *Chaerophyllum*, all species of *Osmorhiza* are very similar, hence there is no real justification for infrageneric division.

Literature Cited

Ball, P. W. 1968. A revision of the genus *Huetia* Boiss. Pp. 3–18 *in* V. H. Heywood (editor), Flora Europaea. Notulae systematicae ad floram europaeam spectantes. No. 7. Feddes Repert. 79.

Bell, C. R. 1971. Breeding systems and floral biology of the Umbelliferae or evidence for specialization in unspecialized flowers. Pp. 93–108 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

Bentham, G. 1867. Umbelliferae. Pp. 859–931 *in* G. Bentham & J. D. Hooker (editors), Genera Plantarum, Vol. 1. Reeve, London.

Boivin, B. 1968. Flora of the Prairie Provinces. Phytologia 17: 57–112.

Calestani, V. 1905. Contributo alla sistematica delle ombrellifere d'Europa. Webbia 1: 89–280.

Candolle, A. P. de. 1829. Mémoire sur la Famille des Ombellifères. Collection de Mémoires pour servir à l'Histoire du Règne Végétal, Vol. 5. Paris.

———. 1830. Umbelliferae. Pp. 55–250 *in* A. P. de Candolle (editor), Prodromus systematis naturalis regni vegetabilis, Vol. 4. Trüttel & Würtz, Paris.

Cannon, J. F. M. 1968. *Anthriscus*. Pp. 326 *in* T. G. Tutin et al. (editors), Flora Europaea, Vol. 2. Cambridge Univ. Press, Cambridge.

Catalán, P., E. A. Kellogg & R. G. Olmstead. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndh*F gene sequences. Molec. Phylogenet. Evol. 8: 150–166.

Cerceau-Larrival, M.-Th. 1962. Plantules et pollens d'Ombellifères. Mém. Mus. Natl. Hist. Nat., sér. B, Bot. 14: 1–166.

———. 1963. Le pollen d'Ombellifères mediterranéennes II. Tordyliinae Drude. Pollen & Spores 5: 297–323.

———. 1965. Le pollen d'Ombellifères mediterranéennes III. Scandicinae Drude. IV. Dauceae Drude. Pollen & Spores 7: 35–62.

———. 1971. Morphologie pollinique et corrélations phylogénétiques chez les Ombellifères. Pp. 109–156 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

Constance, L. 1971. History of the classification of Umbelliferae (Apiaceae). Pp. 1–8 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

——— & R. H. Shan. 1948. The genus *Osmorhiza* (Umbelliferae), a study in geographic affinities. Univ. Calif. Publ. Bot. 23: 111–156.

Crantz, H. J. N. 1767. Classis Umbelliferarum emendata. Leipzig.

Crowden, R. K., J. B. Harborne & V. H. Heywood. 1969. Chemosystematics of the Umbelliferae—A general survey. Phytochemistry 8: 1963–1984.

Czerepanov, S. K. 1995. Vascular Plants of Russia and Adjacent States (the Former USSR). Cambridge Univ. Press, Cambridge.

Dandy, J. E. & J. M. F. Cannon. 1968. *Myrrhoides* Heist. ex Fabr., Enum. Meth. Pl. 37 (1759). Pp. 64–65 *in* V. H. Heywood (editor), Flora Europaea Notulae systematicae ad floram europaeam spectantes. No. 7. Feddes Repert. 79.

Davis, P. H. (editor). 1972. Flora of Turkey and the East Aegean Islands, Vol. 4. Edinburgh Univ. Press, Edinburgh.

Downie, S. R. & D. S. Katz-Downie. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Amer. J. Bot. 83: 234–251.

———, S. Ramanath, D. S. Katz-Downie & E. Llanas. 1998. Molecular systematics of Apiaceae subfamily Apioideae: Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacer and plastid *rpoC1* intron sequences. Amer. J. Bot. 85: 563–591.

———, D. S. Katz-Downie & K. Spalik. 2000a. A phylogeny of Apiaceae tribe Scandiceae: Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Amer. J. Bot. 87: 76–95.

———, ——— & M. F. Watson. 2000b. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: Towards a suprageneric classification of subfamily Apioideae. Amer. J. Bot. 87: 273–292.

———, M. F. Watson, K. Spalik & D. S. Katz-Downie. 2000c. Molecular systematics of Old World Apioideae (Apiaceae): Relationships among some members of tribe Peucedaneae sensu lato, the placement of several island-endemic species, and resolution within the apioid superclade. Canad. J. Bot. 78: 506–528.

Drude, C. G. O. 1898. Umbelliferae. Pp. 63–250 *in* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien, Vol. 3(8). Wilhelm Engelmann, Leipzig.

Engstrand, L. 1973. Generic delimitation of *Bunium*, *Conopodium* and *Geocaryum* (Umbelliferae). Bot. Not. 126: 146–154.

———. 1977. Biosystematics and Taxonomy in *Geocaryum* Cosson (Umbelliferae). University of Lund, Lund.

Gray, A. 1859. Diagnostic characters of new species of phanerogamous plants, collected in Japan by Charles Wright, botanist of the U.S. North Pacific exploring expedition. Mem. Amer. Acad. Arts 6: 377–452.

Greuter, W., J. McNeill, F. R. Barrie, H. M. Burdet, V. Demoulin, T. S. Filgueiras, D. H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland & D. L. Hawksworth. 2000. International Code of Botanical Nomenclature (Saint Louis Code). Regnum Veg. 138.

Grime, J. P. 1988. The C-S-R model of primary plant strategies—Origins, implications and tests. Pp. 371–393 *in* L. D. Gottlieb & S. K. Jain (editors), Plant Evolutionary Biology. Chapman & Hall, London.

———, J. G. Hodgson & R. Hunt. 1988. Comparative Plant Ecology. Unwin Hyman, London.

Guyot, M. 1971. Phylogenetic and systematic value of stomata of the Umbelliferae. Pp. 199–214 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

———, M.-T. Cerceau-Larrival, M.-C. Carbonnier-Jarreau, L. Derouet & J. Relot. 1980. Corrélations entre types stomatiques et types polliniques dans la tribu des Caucalidées (Ombellifères). Bull. Mus. Natl. Hist. Nat., Sér. 4, Misc. 4: 341–385.

Haller, A. von, 1745. Flora jenensis. Jena.

Harborne, J. B. 1971. Flavonoid and phenylpropanoid patterns in the Umbelliferae. Pp. 292–314 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

——— & C. A. Williams. 1972. Flavonoid patterns in the fruits of the Umbelliferae. Phytochemistry 11: 1741–1750.

———, V. H. Heywood & C. A. Williams. 1969. Distribution of myristicin in seeds of the Umbelliferae. Phytochemistry 31: 1729–1732.

Hedge, I. C. & J. M. Lamond. 1972a. *Chaerophyllum* L. Pp. 312–318 *in* P. H. Davis (editor), Flora of Turkey and the East Aegean Islands, Vol. 4. Edinburgh Univ. Press, Edinburgh.

——— & ———. 1972b. *Anthriscus* Pers. Pp. 321–325 *in* P. H. Davis (editor), Flora of Turkey and the East Aegean Islands, Vol. 4. Edinburgh Univ. Press, Edinburgh.

——— & ———. 1972c. *Scandix* L. Pp. 325–330 *in* P. H. Davis (editor), Flora of Turkey and the East Aegean Islands, Vol. 4. Edinburgh Univ. Press, Edinburgh.

——— & ———. 1980. Notes on Umbelliferae: Some Asiatic Scandiceae. Notes Roy. Bot. Gard. Edinburgh 38: 251–257.

——— & ———. 1987. Tribus Scandicinae. Pp. 83–110 *in* I. C. Hedge, J. M. Lamond, K. H. Rechinger et al., Umbelliferae. *In* K. H. Rechinger (editor), Flora Iranica, Vol. 162. Akademische Druck- und Verlagsanstalt, Graz.

———, ———, K. H. Rechinger, et al. 1987. Umbelliferae. Pp. 1–555 *in* K. H. Rechinger (editor), Flora Iranica, Vol. 162. Akademische Druck- und Verlagsanstalt, Graz.

Heywood, V. H. 1971. Systematic survey of Old World Umbelliferae. Pp. 31–42 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

———. 1982. General introduction to the taxonomy of the Umbelliferae. Pp. 107–112 *in* A.-M. Cauwet-Marc & J. Carbonnier (editors), Les Ombellifères "Contributions pluridisciplinaires à la systématique." Actes du 2ème Symposium International sur les Ombellifères. Monogr. Syst. Bot. Missouri Bot. Gard., Vol. 6.

Higgins, D. G., A. J. Bleasby & R. Fuchs. 1992. CLUSTAL V: Improved software for multiple sequence alignment. CABIOS 8: 189–191.

Holmgren, P. K., N. H. Holmgren & L. C. Barnett (editors). 1990. Index Herbariorum. Part I: The Herbaria of the World, 8th ed. Regnum Veg. 120.

Jury, S. L. 1986. Fruit and leaf variation in the African species of the Umbelliferae tribe Caucalideae. Symb. Bot. Upsal. 26: 181–188.

Katz-Downie, D. S., C. M. Valiejo-Roman, E. I. Terentieva, A. V. Troitsky, M. G. Pimenov, B.-Y. Lee & S. R. Downie. 1999. Towards a molecular phylogeny of Apiaceae subfamily Apioideae: Additional information from nuclear ribosomal DNA ITS sequences. Pl. Syst. Evol. 216: 167–195.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Molec. Evol. 16: 111–120.

Koch, W. D. J. 1824. Generum tribuumque plantarum Umbelliferarum nova dispositio. Nova Acta Acad. Caes. Leop., Carol. German. Nat. Cur. 12: 55–156.

Korovin, E. P. 1950. *Albertia* Rgl. et Schm. Pp. 151–152 *in* B. K. Schischkin, Zontitchnye-Umbelliferae Moris. *In* B. K. Schischkin (editor), Flora SSSR, Vol. 16. Izdatelstvo Akademii Nauk SSSR, Moskva, Leningrad.

Koso-Poljansky, B. M. 1916. Sciadophytorum systematis lineamenta. Bull. Soc. Imp. Naturalistes Moscou 29: 93–222.

———. 1920. Zonticnye (Umbelliferae). Pp. 1–120 *in* B. A. Fedtschenko (editor), Flora Aziyatskoy Rosii, Vol. 15. Izd. Glavn. Bot. Sadom, Petrograd.

———. 1923. Systema *Chaerophyllorum* Rossiae. Bot. Mater. Gerb. Glavn. Bot. Sada RSFSR 4(23–24): 189–190.

Kuntze, O. 1891. Revisio Generum Plantarum. Felix, Leipzig.

Lee, B.-Y. & S. R. Downie. 1999. A molecular phylogeny of Apiaceae tribe Caucalideae and related taxa: Inferences based on ITS sequence data. Syst. Bot. 24: 461–479.

Li, H.-L. 1972. Eastern Asia-eastern North America species-pairs in wide-ranging genera. Pp. 65–78 *in* G. Graham (editor), Floristics and Paleofloristics of Asia and eastern North America. Elsevier, Amsterdam.

Linnaeus, C. 1753. Species plantarum. Stockholm.

———. 1762. Species plantarum. Ed. 2. Stockholm.

Lloyd, D. G. 1980. Sexual strategies in plants I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol. 86: 69–79.

———, C. J. Webb & R. B. Primack. 1980. Sexual strategies in plants II. Data on the temporal regulation of maternal investment. New Phytol. 86: 81–92.

Lowry, P. P. & A. G. Jones. 1979. Biosystematic investigations and taxonomy of *Osmorhiza* Rafinesque section *Osmorhiza* (Apiaceae) in North America. Amer. Midl. Naturalist 101: 21–27.

——— & ———. 1984. Systematics of *Osmorhiza* Raf. (Apiaceae: Apioideae). Ann. Missouri Bot. Gard. 71: 1128–1171.

Maddison, W. P. & D. R. Maddison. 1992. MacClade. Analysis of Phylogeny and Character Evolution, Vers. 3. Sinauer, Sunderland, Massachusetts.

Mayr, E. 1982. The Growth of Biological Thought. Belknap Press, Cambridge.

Meusel, H., E. Jäger, S. Rauschert & E. Weinert. 1978. Vergleichende Chorologie der Zentraleuropäischen Flora, Vol. 2. Fischer, Jena.

Morison, R. 1699. Plantarum historiae universalis. Oxford.

Neilreich, A. 1859. Flora von Nieder-Oesterreich. Carl Gerolds Sohn, Wien.

Nielsen, B. E. 1971. Coumarin patterns in the Umbelliferae. Pp. 325–336 *in* V. H. Heywood (editor), The Biology and Chemistry of the Umbelliferae. Academic Press, London.

Pimenov, M. G. & E. V. Kljuykov. 1987. *Neoconopodium*—A new genus of the Umbelliferae from the Himalaya. Feddes Repert. 98: 373–378.

——— & M. V. Leonov. 1993. The Genera of the Umbelliferae. Royal Botanic Gardens, Kew.

Plunkett, G. M., D. E. Soltis & P. S. Soltis. 1996. Evolutionary patterns in Apiaceae: Inferences based on *mat*K sequence data. Syst. Bot. 21: 477–495.

———, ——— & ———. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbc*L sequence data. Amer. J. Bot. 84: 565–580.

Rechinger, K. H. 1952. Umbelliferae novae iranicae. III. Anz. Österr. Akad. Wiss., Math.-Naturwiss. Kl. 11: 240–244.

———. 1987a. *Kozlovia*. Pp. 111–112 *in* I. C. Hedge, J. M. Lamond, K. H. Rechinger et al., Umbelliferae. *In* K. H. Rechinger (editor), Flora Iranica, Vol. 162. Akademische Druck-und Verlagsanstalt, Graz.

———. 1987b. *Rhopalosciadium*. Pp. 130–131 *in* I. C. Hedge, J. M. Lamond, K. H. Rechinger et al., Umbelliferae. *In* K. H. Rechinger (editor), Flora Iranica, Vol. 162. Akademische Druck-und Verlagsanstalt, Graz.

Reduron, J.-P. 1982. Contribution à l'étude morphologique du petale chez les ombellifères. Pp. 121–131 *in* A.-M. Cauwet-Marc & J. Carbonnier (editors), Les Ombellifères "Contributions pluridisciplinaires à la systématique." Actes du 2ème Symposium International sur les Ombellifères. Monogr. Syst. Bot. Missouri Bot. Gard. Vol. 6.

——— & C. E. Jarvis. 1992. 5935. *Chaerophyllum* Linnaeus. P. 560 *in* C. E. Jarvis (editor), Seventy-two proposals for the conservation of types of selected Linnean generic names, the report of Subcommittee 3C on the lectotypification of Linnean generic names. Taxon 41: 552–583.

——— & K. Spalik. 1995. Le genre *Anthriscus* (Apiaceae) dans la flore française. Acta Bot. Gal. 142: 55–96.

Schischkin, B. K. 1950a. Zontitchnye-Umbelliferae Moris. Pp. 36–648 *in* B. K. Schischkin (editor), Flora SSSR, Vol. 16. Izdatelstvo Akademii Nauk SSSR, Moskva, Leningrad.

———. 1950b. *Chaerophyllum* L. Pp. 94–117 *in* B. K. Schischkin, Zonticnye-Umbelliferae Moris. *In* B. K. Schischkin (editor), Flora SSSR, Vol. 16. Izdatelstvo Akademii Nauk SSSR, Moskva, Leningrad.

———. 1950c. *Krasnovia* M. Pop. Pp. 117–118 *in* B. K. Schischkin, Zonticnye-Umbelliferae Moris. *In* B. K. Schischkin (editor), Flora SSSR, Vol. 16. Izdatelstvo Akademii Nauk SSSR, Moskva, Leningrad.

Sheh, M. L. & P. Su. 1987. The floristic analysis of endemic genera in Chinese Umbelliferae. Bull. Nanjing Bot. Gard. 1987: 14–26.

Silvestre, S. 1973. Estudio taxonómico de los géneros *Conopodium* Koch y *Bunium* L. en la Península Ibérica.—II. Parte sistemática. Lagascalia 3: 3–48.

Spalik, K. 1996. Species boundaries, phylogenetic relationships and ecological differentiation in *Anthriscus* (Apiaceae). Pl. Syst. Evol. 199: 13–32.

———. 1997. Revision of *Anthriscus* (Apiaceae). Polish Bot. Stud. 13: 1–69.

Swofford, D. L. 1998. PAUP*. Phylogenetic Analysis Us-

ing Parsimony (*and other methods). Vers. 4. Sinauer, Sunderland, Massachusetts.

Tausch, I. F. 1834. Das System der Doldengewächse. Flora 17(22): 337–357.

Thellung, A. 1926. *Umbelliferae*. Pp. 926–1537 *in* G. Hegi (editor), Illustrierte Flora von Mittel-Europa, Vol. 5(2). Hauser, München.

Tutin, T. G. 1968. Notes on the genus *Athamanta* (Umbelliferae) in Europe. Pp. 18–20 *in* V. H. Heywood (editor), Flora Europaea. Notulae systematicae ad floram europaeam spectantes. No. 7. Feddes Repert. 79.

———, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters & D. A. Webb (editors). 1968. Flora Europaea, Vol. 2. Cambridge Univ. Press, Cambridge.

Vinogradova, V. M. 1999. The type specimens of the Apiaceae taxa from the Middle Asia kept in the Herbarium of the Komarov Botanical Institute (LE). 3. *Aegopodium—Eryngium*. Bot. Zhurn. 84: 80–87.

Webb, C. J. 1981. Andromonoecism, protandry, and sexual selection in Umbelliferae. New Zealand J. Bot. 19: 335–338.

Appendix 1. Characters used in the morphological analysis. Arrows indicate ordinal characters, the remaining are multistate or binary.

Life History and Vegetative Morphology

1. Reproductive strategy: 0, always monocarpic ↔ 1, monocarpic or polycarpic ↔ 2, always polycarpic. An ability to switch between monocarpy and polycarpy represents a distinct life history strategy; therefore it was coded as an intermediate state rather than a polymorphism. This feature is often reflected in plant habit. For instance, *Anthriscus sylvestris* subsp. *sylvestris* has monocarpic biennial rosettes but may perennate by buds in the axils of the basal leaves (Grime et al., 1988).
2. Root: 0, not tuberous; 1, tuberous; 2, globose tuber. Geophytes (i.e., plants with globose tubers) can easily be distinguished in herbarium material even if the tuber is not preserved due to the presence of thin, flexuose bases of the leaf stalks and stems. Species with tuberous roots are usually hemicryptophytes and neither their stems nor leaf stalks have flexuose bases.
3. Leaf division: 0, leaf not divided ↔ 1, 1-pinnate ↔ 2, 2-pinnate ↔ 3, 3-pinnate ↔ 4, 4-pinnate. There is variation in leaf division within each species and even on a single plant; therefore, the most common state for each taxon was taken. This character is diagnostic at the infrageneric level; for instance, to separate subgenus *Osmorhiza* from subgenus *Glycosma* or, in *Anthriscus*, to distinguish *A. lamprocarpa* from the closely related *A. sylvestris*.
4. Lobe orientation: 0, leaf ± flat; 1, lobes somewhat spreading; 2, lobes radially spreading around the axis.
5. Shape of basal leaf lobes: 0, ovate to lanceolate; 1, linear-lanceolate to linear.
6. Shape of cauline leaf lobes: 0, ovate to lanceolate; 1, linear; 2, leaves reduced to sheaths. Cauline and basal leaves are usually similar, although the former may have broader lobes. However, in some species (e.g., *Chaerophyllum macrospermum* and *C. macropodum*) the lobes of the cauline leaves, particularly of the uppermost leaves, are morphologically distinct from those of the basal leaves.

Bracts and Bracteoles

7. Bracts: 0, absent or single ↔ 1, few ↔ 2, numerous.
8. Bract margin: 0, entire; 1, pinnate. Bracts and bracteoles are reduced cauline leaves, and hence their morphology often reflects the leaf morphology. If they are reduced to leaf sheaths, they are entire; if the leaf blade is present, they may be incised or pinnate. If bracts are absent, the character was coded as inapplicable.
9. Number of bracteoles: 0, bracteoles absent ↔ 1, 1–3 ↔ 2, 4–5 ↔ 3, more than 5. The number of bracteoles is usually more or less constant, but in *Heteromorpha* there is much variability so this character was coded as missing. If bracteoles are absent, characters 10–13 were coded as inapplicable.
10. Bracteole division: 0, bracteoles entire (not incised) ↔ 1, incised ↔ 2, pinnate.
11. Bracteole margin: 0, deprived of cilia or scales ↔ 1, with minute scales ↔ 2, ciliate. Cilia at bracteole margins are homologous to those at leaf sheath margins and are distinctly longer than hairs that may cover the bracteole. However, in some species with densely hairy bracteoles it was impossible to determine the state of this character and, therefore, it was coded as "0 or 2."
12. Bracteole indumentum: 0, absent; 1, present.
13. Bracteole shape: 0, ovate to narrow lanceolate; 1, linear.

Inflorescence

14. Number of umbellets in primary umbel: 0, less than four; 1, more than four. The number of umbellets (rays) in a primary umbel is variable, but in species with reduced primary umbels it rarely reaches four while in those with well-developed primary umbels it often exceeds ten.
15. Umbel width: 0, more than 2 cm diam.; 1, less than 2 cm diam.
16. Umbel position: 0, mostly terminal ↔ 1, terminal and lateral ↔ 2, mostly lateral. Terminal versus lateral umbel formation is an important element of plant reproductive strategy. Species with terminal umbels are characterized by determinate flowering, and hence the number of consecutive orders of umbels rarely exceeds four (Bell, 1971) and the regulation of reproductive effort is predominantly postgamic (Lloyd, 1980; Lloyd et al., 1980). Lateral formation of umbels allows indeterminate flowering, i.e., further growth of the main axis and the development of additional orders of umbels when resources are abundant. A good example of such a strategy is found in *Anthriscus caucalis*, which may have up to nine orders of umbels permitting a more flexible pregamic regulation of reproductive effort (Spalik, 1996).
17. Peripheral flowers: 0, perfect; 1, male. The majority of umbellifers are andromonoecious, i.e., they have both hermaphrodite and male flowers, the latter usually situated inside the umbellet although the opposite may also be found. Characters 17–20 were determined from primary or secondary umbels. In andromonoecious species umbels of higher orders may bear only male flowers.
18. Disc male flowers: 0, present; 1, absent.
19. Central flower: 0, male or similar to other flowers; 1, perfect, sessile or with distinctly shorter pedicels than other perfect flowers.

20. Outer flowers: 0, actinomorphic or slightly zygomorphic; 1, distinctly zygomorphic.

Floral Morphology

21. Sepals: 0, present; 1, reduced; 2, absent. It is sometimes difficult to determine whether small projections at the fruit top are indeed reduced calyx teeth, as in *Myrrhis*. This character was therefore coded as unordered rather than ordinal.
22. Sepal indumentum: 0, absent; 1, present.
23. Petal incision: 0, absent or shallow; 1, deep. Deeply incised petals were regarded as cut to at least one-third of their length. This character is, however, quite variable since species with zygomorphic flowers have outer petals deeply cut while their inner petals are entire. In such cases, only outer petals were considered.
24. Petal margin: 0, naked ↔ 1, denticulate ↔ 2, ciliate. Small teeth at petal margin seem to be reduced cilia, therefore this character was coded as ordinal.
25. Petal indumentum: 0, absent; 1, present.
26. Oil ducts in petals: 0, present; 1, absent. Since petals were not dissected, it was sometimes difficult to determine whether dark-colored lines seen at the outer sides of petals were oil ducts or veins. Therefore, this character was scored as missing for some species.

Fruit Morphology

27. Stylopodium shape: 0, flat-conic; 1, high-conic; 2, ovoid or rounded. The shape of the stylopodium changes as the fruit matures, and thus its shape was determined at fruit maturity.
28. Crown of hairs (bristles, scales) at fruit base: 0, absent; 1, present. Hairs (bristles, scales) forming the crown on the pedicel at the base of the fruit, if present, are distinctly longer than those that may occur below (character 29). This crown of hairs may be reduced to single scales; if so, the pedicel is usually naked.
29. Pedicel indumentum: 0, absent; 1, pedicels scabrid; 2, pedicels hairy. Scabrid pedicels denote small dents that may occur on their inner sides.
30. Pedicels of fruit: 0, not thickened; 1, thickened. When thickened at fruit maturity, pedicels may obtain the diameter of fruits.
31. Fruit shape: 0, globose ↔ 1, elliptic or ovate to broadly ovate ↔ 2, narrow to oblong ovate ↔ 3, linear-oblong. The order of states reflects the increasing proportion of fruit length to width.
32. Fruit appendage: 0, absent; 1, present. A pedicel-like appendage is formed if the seed does not fill the lower part of the fruit. It is characteristic only for *Osmorhiza*, with the exception of *O. occidentalis*.
33. Commissure: 0, broad; 1, constricted.
34. Beak: 0, obsolete ↔ 1, relatively short ↔ 2, long. A distinct beak is formed when the seed does not fill the top of the fruit. It is sometimes a different color than the rest of the fruit.
35. Primary ridge shape: 0, arched or obsolete; 1, filiform to nearly winged; 2, broad and rounded; 3, angular only at top, obsolete below; 4, angular throughout. As arched we denote broad semicircular ridges that touch each other and are not separated from vallecules—sometimes such ridges are not much pronounced and may be regarded as obsolete. Narrow, filiform ridges are usually of a different color than the vallecules and may form small wing-like projections. Broad and rounded ridges, such as those occurring in *Chaerophyllum*, are also of a different color than the vallecules. Some fruits (their upper portion or throughout) are pentangular or even star-shaped in transverse section.
36. Primary ridge indumentum: 0, absent; 1, hairs or bristles. As primary ridge indumentum we consider only those hairs or bristles that are distinctly lined along the ridges. Therefore, in taxa with hairs or bristles evenly scattered over the fruit surface (e.g., *Anthriscus sylvestris* subsp. *nemorosa*), this character is regarded as absent.
37. Mericarp compression: 0, not compressed (i.e., mericarp as broad as wide) ↔ 1, somewhat dorsally compressed ↔ 2, distinctly dorsally compressed. Fruits lacking compressed mericarps are also described as laterally compressed.
38. Secondary ridges: 0, absent; 1, present.
39. Secondary ridge appendages: 0, wings; 1, spines; 2, hairs. If secondary ridges are absent, this character was coded as inapplicable.
40. Tubercles at fruit surface: 0, absent; 1, present. Tubercles usually constitute the bases of bristles. As these sometimes break away easily, only tubercles can remain on herbarium material, and hence the fruits can be described as tuberculate rather than bristled.
41. Fruit indumentum: 0, absent; 1, hairs; 2, bristles. In *Krasnovia longiloba* tubercles lack normally developed bristles but sometimes end with short hyaline teeth that seem to be homologous with bristles. Therefore, for this species, the character was coded as polymorphic. *Anthriscus caucalis* and *A. cerefolium* include varieties with both naked and bristled fruits.
42. Cuticle appearance: 0, dull; 1, shiny. This character is difficult to determine in badly preserved herbarium material or when the fruits are not fully ripe. However, it does reflect an anatomical basis (i.e., shiny fruits are usually characterized by a thick cuticle while those with a rather dull appearance have a thin cuticle; K. Spalik, A. Wojewódzka & S. Downie, unpublished data).
43. Cuticle texture: 0, smooth or striate; 1, aculeate. Small projections that give an aculeate texture to the cuticle are formed above the centers of the cells. In *Scandix*, they are found only close to the fruit base, while in *Anthriscus lamprocarpa* they may occur only near the commissure.
44. Epidermis coloration: 0, uniform (not areolate); 1, areolate. The areolate appearance of epidermis is due to thickened transverse cell walls (Spalik, 1997).

Appendix 2. Matrix of morphological data. Character numbers refer to those presented in Appendix 1. Question marks denote missing data or polymorphisms that include all character states; hyphens denote inapplicable characters: A, B, and C denote character states "0 or 2," "0 and 1," and "0 and 2," respectively.

	Character number				
	1	1111111112	2222222223	3333333334	4444
Taxon	1234567890	1234567890	1234567890	1234567890	1234
Anthriscus caucalis	0040000020	2000020100	2-00010101	20113000-1	C111
Anthriscus cerefolium	0030000010	2000010001	2-00010000	30113000-1	C111
Anthriscus kotschyi	2030000010	0000000000	2-00010000	20113000-0	0111
Anthriscus lamprocarpa	0020000020	2000000001	2-00010100	20113000-0	0111
Anthriscus nitida	1030000020	2000000001	2-00010100	20113000-0	0111
Anthriscus schmalhausenii	2020000020	2000000001	2-00010100	20113000-0	0111
Anthriscus sylvestris subsp. *sylvestris*	1040000020	2000000001	2-00010100	20113000-0	0111
Anthriscus sylvestris subsp. *fumarioides*	2040000020	2000000001	2-00010100	20113000-1	2111
Anthriscus sylvestris subsp. *nemorosa*	2040000020	2000000001	2-00010100	20113000-1	2111
Apium graveolens	0010000-0-	---1010100	2-00010000	20001000-0	0000
Athamanta cretensis	2031111030	2101000010	0100102020	20111100-0	1000
Athamanta della-cellae	2010002030	A101000000	0110112020	20111100-0	1000
Athamanta sicula	2041101030	2101000000	0102102020	20111100-0	10?0
Athamanta turbith subsp. *turbith*	2031111030	2101000110	0100102020	20111100-0	1000
Balansaea glaberrima	2220010020	0001000010	2-000?2000	20111000-0	0000
Bubon macedonicum subsp. *macedonicum*	2030001020	A111000000	0100102020	10011110-0	1000
Bunium elegans	2220112030	0000000100	2-10001001	20001000-0	0000
Carum carvi	2021000000	0011000001	00100?0000	10102000-0	0000
Caucalis platycarpos	0030100010	2000020000	0010001111	1011110111	2000
Chaerophyllopsis huaui	?030000-0-	---?000000	00000?0000	10101000-0	0000
Chaerophyllum aromaticum	2020000030	2101000011	2-10011000	20012000-0	0000
Chaerophyllum astrantiae	2021110020	2101000011	2-10010000	30012000-0	0000
Chaerophyllum atlanticum	2020001030	2101000010	2-10111000	30002000-0	0000
Chaerophyllum aureum	2030000020	2101000011	2-10011000	20012000-0	0000

Appendix 2. Continued.

Chaerophyllum azoricum	2020000030	2101000011	2-10010000	30002000-0	0000
Chaerophyllum bulbosum	0130010020	0001000011	2-10012000	20012000-0	0000
Chaerophyllum byzantinum	2020001030	2001000011	2-10011000	30002000-0	0000
Chaerophyllum crinitum	0131110020	2001000011	2-11011000	30012000-0	0000
Chaerophyllum elegans	2030000020	2001000011	2-12012000	30012000-0	0000
Chaerophyllum hirsutum	2030000020	2001000011	2-12012000	30012000-0	0000
Chaerophyllum khorassanicum	2040000020	2100000011	2-10111020	30002000-0	1000
Chaerophyllum libanoticum	2020001030	2001000011	2-10011000	30002000-0	0000
Chaerophyllum macropodum	0041010020	2100000011	2-10110001	30002000-0	0000
Chaerophyllum macrospermum	1031010030	1100000011	2-10111000	30002000-0	0000
Chaerophyllum magellense	2020000030	2101000011	2-12011000	30012000-0	0000
Chaerophyllum meyeri	0120000020	0001000011	2-10010000	20012000-0	0000
Chaerophyllum procumbens	0020000020	2000020110	2-00010000	20012000-0	B000
Chaerophyllum tainturieri	0031000020	1000020110	2-00010000	10012000-0	B000
Chaerophyllum temulum	0030000020	2101010011	2-10012000	20012000-0	B000
Chaerophyllum villarsii	2020000020	2001000011	2-12012000	30012000-0	0000
Chaetosciadium trichospermum	0020000030	2010110000	0110111020	2010110120	1110
Cicuta virosa	2020000030	0011000100	0000010000	0000A000-0	0000
Conopodium ramosum	2220110010	0011000010	2-000?2000	20111000-0	0000
Daucus carota	0020012132	2101000001	0010010110	1001110111	2000
Echinophora tenuifolia subsp. *sibthorpiana*	1020001020	A100011010	01001?202-	20010100-0	1000
Elaeosticta allioides	2121112030	0001000100	2-10001000	00100000-0	0000
Falcaria vulgaris	0010002020	0011000000	0000011000	20100000-0	0000
Geocaryum macrocarpum	2220010020	1001000001	2-00010100	20114000-0	0110
Grammosciadium daucoides	2022112122	0011000001	0010001000	30002010-0	0000
Grammosciadium macrodon	2022112122	1011000001	0010001000	30002010-0	0000
Grammosciadium platycarpum	2022112112	0011000001	0010001000	30002010-0	0000
Grammosciadium pterocarpum	2012112112	0011000001	0010001000	30002010-0	0000
Heracleum sphondylium	1010000010	2111000001	2-00000020	10001020-0	0000
Heteromorpha arborescens	201000207	A101000000	0000001010	10101000-0	1000

Kozlovia paleacea	1220000020	2001000011	2-10010100	20114100-1	2111
Krasnovia longiloba	2220110020	1001000010	2-00010100	20014000-1	C111
Laserpitium hispidum	1030002030	2101000000	0100010020	1000110100	2000
Laserpitium petrophilum	2020012030	2001000000	0000010010	1000110100	2000
Lecokia cretica	2020001030	0011000000	0000001010	10100000-0	2000
Myrrhis odorata	2030000020	210-000001	1010011020	20114100-0	2101
Myrrhoides nodosa	0020000020	2100020010	2-101?0021	20002000-1	2000
Neoconopodium capnoides	2220000020	1001000001	2-00010000	20114B00-0	C111
Neoconopodium laseroides	2220000020	1001000001	2-00010100	20114100-0	2111
Orlaya grandiflora	0020001010	1001000001	0010000101	1001111110	2000
Osmorhiza aristata	2030000020	2100000001	2-01011100	31114100-0	2111
Osmorhiza berteroi	2030000000	2000000000	2-01012100	31114100-0	2111
Osmorhiza brachypoda	2030001020	2000000000	2-02010000	31114100-0	2111
Osmorhiza claytonii	2030000020	2000000000	2-01011100	31114100-0	2111
Osmorhiza depauperata	2030000000	2001000000	2-01012100	31114100-0	2111
Osmorhiza longistylis	2030001020	2000000001	2-01011100	31114100-0	2111
Osmorhiza mexicana subsp. *bipatriata*	2030000010	2000000000	2-01010100	31114100-0	0111
Osmorhiza mexicana subsp. *mexicana*	2030000010	2000000000	2-01010100	31114100-0	2111
Osmorhiza occidentalis	2020000000	2001000000	2-01010000	30114100-0	0111
Osmorhiza purpurea	203000000-	---0000000	2-01010100	31114100-0	2111
Pastinaca sativa	0010000010	0011000000	2-00010010	10001020-0	0000
Petroselinum crispum	0030001130	0011000000	2-00100000	10001000-0	0000
Pimpinella peregrina	0010010000	A111000100	2-00002020	10001100-0	10?0
Pseudorlaya pumila	0020001111	2100010000	0100101020	1000111110	2000
Rhabdosciadium aucheri	2000021020	0001121010	0000001000	30101000-0	0000
Rhopalosciadium stereocalyx	0020000020	A11?000???	011011102?	30?0?1???0	2???
Scaligeria moreana	0020020020	0011000100	2-10001000	00100000-0	0000
Scandix balansae	0021110021	1000010001	2-00010001	20022100-0	2011
Scandix iberica	0021110021	2100010001	2-10010001	20022100-0	2011
Scandix pecten-veneris	0021110021	2000010001	2-00011001	30022100-1	2011

Appendix 2. Continued.

Sium latifolium	2010002030	0001000000	0000012010	10001000-0	0000
Smyrnium olusatrum	0030000020	1001000011	10000?2010	10101000-0	0000
Sphallerocarpus gracilis	0040000020	2001000001	1000010000	10101000-0	0000
Tinguarra cervariifolia	2030001030	2111000000	0102102020	10111100-0	10?0
Tinguarra montana	2040000030	2111000000	0102102020	10101110-0	1000
Torilis nodosa	0020001010	A110120100	0000112021	1010110111	2-10

THE GENUS *HYPOXIS* (HYPOXIDACEAE) IN CENTRAL AFRICA[1]

Justyna Wiland-Szymańska[2]

Abstract

Hypoxis is a pantropical genus, with about 100 species worldwide. It is represented in Central Africa by 20 species, with 3 species in Rwanda, 5 in Burundi, and 19 in Congo-Kinshasa. This study considers morphology of vegetative and reproductive organs with emphasis on leaves, inflorescences, seeds, and indumentum. Morphology and anatomy of trichomes and other new data on taxonomically useful characters of *Hypoxis* in Central Africa are assessed.

Key words: Africa, anatomy, Hypoxidaceae, *Hypoxis*, morphology.

Morphological variability of *Hypoxis* L. confuses its taxonomy and nomenclature. Established by Linnaeus (1759), *Hypoxis* has often been divided into two infrageneric taxa, one including plants with indumentum and the other including glabrate species. The first has been described as *Hypoxis* subg. *Euhypoxis* Baker (1878b: 99), *Hypoxis* sect. *Euhypoxis* (Baker) Benth. (Bentham & Hooker, 1883: 717), or *Hypoxis* sect. *Hypoxis* (Geerinck, 1969: 75).

Salisbury (1866) first described the second group as two distinct genera, *Ianthe* Salisb. and *Spiloxene* Salisb., to which almost glabrous South African plants belong. Baker (1878b: 99) treated both *Ianthe* and *Spiloxene* as synonyms of *Hypoxis* subg. *Ianthe* (Salisb.) Baker. Subsequently Williams (1901) and Nel (1914a, b) recognized *Ianthe* as a separate genus (Nel misspelled it as *Janthe* Salisb.), and both of them explicitly included *Spiloxene* in its synonymy. Their taxonomy was endorsed by Hilliard and Burtt (1978), but they mistakenly used *Spiloxene* as the name of the genus, a usage not permitted under ICBN Article 11.5 (Greuter et al., 2000: 21). In this paper I recognize *Hypoxis* and *Ianthe* as separate genera, and therefore the description given is of *Hypoxis* sensu stricto (excluding *Ianthe*).

No recent thorough study of *Hypoxis* exists for Central Africa, consisting herein of Democratic Republic of Congo (= Congo-Kinshasa), Rwanda, and Burundi. This paper is based mainly on morphological characters, though apomixis and pseudogamy have been confirmed for some African species (Wilsenach, 1967; Wilsenach & Papenfus, 1967; Wilsenach & Warren, 1967; Zimudzi, 1994). Molecular studies of all *Hypoxis* species from tropical Africa, as delimited by Lebrun and Stork (1991), are planned for the future.

Materials and Methods

Approximately 300 herbarium sheets from B, BM, BR, BRLU, K, KRA, KRAM, MO, O, P, POZG, UPS, WAG, and Z were used for morphological and biometric studies (Index to Exsiccatae follows the Literature Cited). Flowers from herbarium specimens were boiled in water for morphological analysis under a dissecting microscope. Trichomes were taken from boiled scapes and leaves and examined using ordinary and polarizing light microscopy, with drawings made from the light microscope. Freeze-dried seeds and also some trichomes were examined using scanning electron microscopy after coating with gold. Data on ecology and distribution were taken from the herbarium labels. Distribution data in the text are presented in the original languages.

Results and Discussion

Of the 20 species of *Hypoxis* known from Central Africa, 19 occur in the southern and eastern parts of Congo-Kinshasa, with only 1 species, *H. angustifolia* Lam., extending into the western part of the country. Only 3 species occur in Rwanda and 5 in

[1] Support of this study by a grant from the Polish Science Research Committee is gratefully acknowledged. I thank the curators and workers of the herbaria that I visited (B, BM, K, KRA, KRAM, O, P, UPS, Z) for their help and hospitality, and especially those at MO. I am very grateful to the curators of B, BR, BRLU, and WAG for the loan of herbarium material. My special thanks are addressed to Stanisław Lisowski, Dept. of Geobotany, A. Mickiewicz University, Poznań, for supervising my Ph.D. study, and Inger Nordal, University of Oslo, for her help and kindness. I thank Roy Gereau, as well as the editors, David Bogler, and anonymous reviewers for helpful comments and suggestions during preparation of the manuscript. Most illustrations were drawn by Marcin Matuszczak.

[2] Department of Geobotany, Adam Mickiewicz University, Al. Niepodległości 14, 61-713 Poznań, Poland.

Burundi. The largest number of taxa in Congo-Kinshasa (18) is found in the Shaba Province. This situation is a consequence of two factors. First, southern and eastern parts of Congo-Kinshasa are covered with wooded grassland, the habitat in which most species of the sun-loving genus *Hypoxis* grow. Many species of the genus are characteristic floristic elements of the Zambezian regional center of endemism (White, 1983). Second, Shaba is a center of endemism itself, in part due to altitudinal and edaphic diversity, with local areas of soils with high levels of heavy metals (Duvigneaud, 1958; Duvigneaud & Denaeyer-De Smet, 1963; Malaisse, 1983; Mandango & Ndjele, 1994).

Nel (1914a) divided the African species of *Hypoxis* into 11 sections on the basis of variation in four features: morphology of anther apex, relative lengths of style and stigma, leaf width, and number of nerves in leaf blades. Unfortunately, these sections are not of much practical value. Characters of the gynoecium are not stable in polymorphic species, as, for example, *H. angustifolia* and *H. hockii* De Wild. The width of the leaf lamina depends on the age of the leaf, as well as the age of the plant, and it must be specified whether inner or outer leaves are measured. For these reasons I did not assign Central African species to Nel's sections. My studies led me to the conclusion that seed and indumentum characters are the most useful for determining taxonomy and relationships within this genus.

Dimensions and shapes of seeds are similar in all Central African species. Seeds are usually ovoid or almost spherical in shape, and 1 to 2 mm in diameter. The seed coat of *Hypoxis* is encrusted with phytomelan and, therefore, black (Oganezova, 1995). Cells are isodiametric, 5–7-gonal, with straight boundaries (Figs. 3B, 10E, F, 14B & D, 16B, 19B). The anticlinal cell boundaries may be slightly raised (Figs. 10E, F, 14A, B & D, 19B) or channeled (Figs. 3B, 10F, 12B, 16B). Outer periclinal walls of cells may be slightly sunken (Fig. 14B), flat (Figs. 10F, 14D), concave (Fig. 2A), or more or less convex (Figs. 2C, D, 3, 5, 7, 10A–E, 12, 16, 17, 20). In the micropylar part of the seed the outer periclinal walls tend to be more raised than in other parts of the seed (Figs. 10D, 14A). The raised convex papillae may be semispherical (Figs. 3B, 12, 16B), conical (e.g., Figs. 2D, 7D, 10C, 17B, 20D), or nipple-shaped and aculeate (Fig. 10E). The flat or concave papillae are usually smooth in appearance, except *H. hockii* var. *colliculata* Wiland, where they are micropapillate (Fig. 10F), a feature visible even under the dissecting microscope.

The cuticle covering seed coat cells may be very thin (Oganezova, 1995) or very thick in some species (Nordal et al., 1985). In the former the seeds are black, in the latter red-brown or brown due to cuticle coloring. In *H. angustifolia* the thick cuticle is iridescent, as is common in some American *Hypoxis* species (Brackett, 1923). The Central African species fall into two main groups according to seed coat differences: 11 species with brown seeds with a thick layer of cuticle and 8 species with black seeds and a thin layer of cuticle. If the cuticle is thin, it smoothly covers the cell surface. Under high magnification (×625), however, micropapillation is visible (Fig. 16B). The thick cuticle is usually highly wrinkled (Figs. 2C, 3B, 5B, D, 7B, D, 10A, 12D, 13B, 20B, D), and it creates small wings along papillae, so they look like more or less elongated pyramids. The only exception is the seed coat of *H. muhilensis* Wiland subsp. *muhilensis*, where the thick cuticle covers the papillae smoothly, but forms elongated appendages at their apexes (Fig. 17B).

Brackett (1923) and McVaugh (1989) extensively used seed coat structure in keys to the American species of *Hypoxis*. Nordal et al. (1985) and Zimudzi (1996) applied this character to identification of the East African and Zambesian species, respectively. Because it is not known how many genes are expressed in the development of different kinds of seed coat in *Hypoxis*, some researchers (Britt, 1967) believe that they do not constitute differences great enough to warrant separation into distinct species. However, as stated by Barthlott (1981), differences of surface characters always seem to be based on genetic diversity and may have taxonomic importance. In this treatment of Central African *Hypoxis*, seed coat sculpture was evaluated according to its correlation with other morphological characters useful in the delimitation of species. In *H. hockii* three different types of seed coat sculpture occur. It is not possible to state now whether this seed coat heteromorphism is correlated with anything other than genetic polymorphism in this morphologically highly variable species. Because there is no visible correlation with any other factor, the rank of variety is used. In other cases in which there are more supportive characters, seed coat sculpture is used as an additional feature in delimitation of species or subspecies.

All Central African species of *Hypoxis* have vegetative and reproductive organs covered with indumentum. Because *Hypoxis* species occur in areas with a long dry season, the structure of some of their organs, for example the rhizome or roots,

shows xeromorphic features. Some authors (e.g., Nel, 1914a) consider the indumentum of these plants to be an adaptation to minimize transpiration rates. In some species the trichomes fall off with age and as a result some organs become nearly glabrous.

Trichomes of the Central African species of *Hypoxis* have a characteristic structure. Trichome branches grow out of a base (foot) consisting of 4 to 8 cells of an oval or irregular shape. These cells are very well distinguished because they stand out from the surrounding epidermal cells and contain a large, ovoid, dark cell nucleus. Trichome branches do not enclose any cytoplasm, and only remnants of transverse cell walls can be observed. The trichome branches have so far been considered unicellular; however, when examining the cell wall it is easy to note several protuberances on its surface, which are the remains of the transverse walls of the cells that form the branch. They are particularly visible by polarized light, but hardly noticeable under an unpolarized light microscope. This is the reason for the lack of transverse walls in the illustrations. The thickness of the cell walls varies in the individual taxa. Likewise, the hollow core has a variable diameter and may reach the branch apex (Figs. 8H–J, 9E, F, I, J, 18G) or only occur in its basal part (Fig. 15C). The trichome branches are slightly distended in the basal part; in the upper part they become gradually narrower. In some taxa the trichome branches show a tendency to twist (Fig. 1E, F). The branch length ranges from 0.1 to 5.3 mm.

Within Central African *Hypoxis* species there are three types of trichomes distinguished on the basis of the number of branches:

a. Simple trichomes observed on the margin of bracts in *H. urceolata* Nel (Fig. 18C) and *H. angustifolia*; they may rarely occur together with two-branched trichomes on the leaf surface;

b. Two-branched trichomes with base bearing two many-celled branches (Fig. 18I); usually one branch is longer than the other; two-branched trichomes occur most frequently on leaves, but may also appear in the inflorescence;

c. Tufted trichomes with base bearing 3 to 13 many-celled branches; branches differ in length, with one branch always much longer than the others; exceptionally, two longer branches may occur; tufted trichomes may occur on all organs (Figs. 1E, F, 8I, J, 9E, F, I, J, 11D, E, 13C, 15C, D, 18G).

One or two trichome types may occur on one plant. Almost all species have most parts of scapes, pedicels, and flowers covered with tufted trichomes. Two-branched or simple trichomes on these organs are rare and usually correlated with scarce indumentum on the leaves.

Although some trichome and indumentum types are species-specific, there are patterns common to the whole genus. Indumentum is distributed on both the inner and outer leaves. In *H. lejolyana* Wiland, trichomes are present on the tunic. Outer leaves are usually covered with trichomes on the whole blade surface below and more sparsely so above. The trichome topography on the inner leaves is more variable and can be divided into the three following types:

1. Trichomes present on the entire upper and lower surface of the leaf (e.g., *H. urceolata*).

2. Trichomes on the margins, the midrib beneath, and on the surface of the lamina above or below (Fig. 4).

3. Trichomes distributed only on the margins of the blade and on the midrib below (Fig. 1A).

The trichomes on the compressed scape of *Hypoxis* are irregularly distributed. The lower part of the scape is ciliate, but toward the middle sporadic trichomes occur on the surface of the scape, and in the uppermost part the indumentum becomes dense, especially in the pedicel zone. Bracts are covered with indumentum only on the abaxial (outer) side with the trichomes usually restricted to the midrib, but sometimes they may also be present on the lamina, especially toward the base (Fig. 1B). In *H. urceolata*, *H. goetzei* Harms, and *H. angustifolia*, margins of the bract may be ciliate with single- or two-branched trichomes (Figs. 8C, 18C). Bracts in the basal part of the inflorescence are larger and with more trichomes. Pedicels and ovaries usually have indumentum as dense as the upper part of the scape. The outer tepals have a dense indumentum on the entire abaxial surface (Figs. 1A, 4, 11B), and sometimes have a protruding row of perpendicular trichomes along the midrib below. The inner tepals are almost glabrous except for the abaxial midrib (Figs. 8A, 18A), which bears trichomes extending from the segment base toward the apex and covering from ⅓ to its entire length. Additionally, on the adaxial surface of the tepals one may observe single-celled papillae that are pyramidal and larger on the inner tepals than on the outer.

The overall appearance of a plant depends on the indumentum density, its position relative to the organ surface, and color. Trichomes may be hyaline,

grayish, yellowish, golden, or red-brown. Within some species, indumentum has variable colors, for example, different on the outer and inner leaves, but normally the color for an individual taxon is consistent. The indumentum color may also vary with age. Moreover, Nel (1914a) reported transitions of the indumentum color on one individual of South African *H. sobolifera* Jacq. In the Central African species of *Hypoxis*, different types of indumentum occur: villous (*H. lusalensis* Wiland), tomentose (leaves of *H. bampsiana* Wiland), pilose (*H. angustifolia*), hispid (*H. angolensis* Baker), pubescent (*H. hockii* var. *hockii*), or ciliate (leaves of *H. goetzei*). Indumentum density ranges from very compact and dense to sparse or sporadic.

Studies on trichomes of *Hypoxis*, first done by Scharf (1892) and subsequently extended by Nel (1914a), Heidemann (1983), and Nordal et al. (1985), focused mainly on eastern and southern African taxa. Scharf (1892) and Nel (1914a) tried to explain the process of trichome formation, but despite performing their studies on the same South African species (*H. stellipilis* Ker-Gawl.), their observations differed. Whereas Scharf studied the structure of trichomes on the scape, Nel concentrated on the leaves, which have a different trichome type. More recently, Hummel and Staesche (1962), Heideman (1983), and Nordal et al. (1985) discussed a simplified trichome structure in the genus *Hypoxis*. Heideman (1983) and Nordal et al. (1985) presented the trichome foot composed of only one cell with unicellular branches. Hummel and Staesche (1962) mentioned that Hypoxidaceae have many-celled, tufted trichomes. The trichome topography on *Hypoxis* plants was considered a systematic feature with reference to the South African (Heideman, 1983) and East African (Nordal et al., 1985) species. In the latter work, the authors suggested six types of trichome distribution on the leaves, but these types do not include all the variability observed in Central African species, e.g., *H. bampsiana*. The various features of trichomes and indumentum have proven to be useful in creating the keys to the Central African taxa of *Hypoxis*.

In the studied material there were a few specimens that could not be included in any of the species treated below. Their taxonomic status is currently being examined and will be reported in a later publication. Due to the political situation in Central Africa, there are currently no possibilities of performing experimental biological tests in situ, and access to living material is limited as well. Consequently, the current paper presents α-taxonomy based on available data. More studies concerning apomixis and pseudogamy of *Hypoxis* in Central Africa and in other parts of the continent are needed, as well as evaluation of a species concept in this genus.

Hypoxis L. Syst. Nat. ed. 10. 1759. TYPE: *Hypoxis hirsuta* (L.) Colville (syn.: *H. erecta* L.).

Herbs with perennial rhizomes or corms; underground parts globose, ovoid or elongate, yellowish or whitish inside, usually topped by a tunic of membranous and/or fibrous remains of old leaves; roots thick, ribbon-like, contractile. *Leaves* in a basal rosette, often tristichous, usually diversified into outer leaves, developing at beginning of growing season, and inner leaves developing later, both with parallel nervation; *outer leaves* wider and shorter than inner leaves, in basal part usually spathe-like, with membranous margins, enclosing bases of the inner leaves in long or short pseudostem, in upper part ovate, lanceolate, ensiform, linear or canaliculate, acute at apex, often keeled, erect or reflexed, covered with indumentum; *inner leaves* ovate, lanceolate, ensiform, linear or canaliculate, acute at apex, often keeled, erect or slightly reflexed, ± covered with trichomes. *Scapes* compressed, usually tapering toward base, winged and ciliate in lowest part, wider and more covered with trichomes in upper part; *flowers* single or in determinate or indeterminate inflorescences; *bracts* subulate or ovate in basal part, acute, keeled, those subtending lowermost flowers larger than in upper part of inflorescence; *pedicels* ± distinct, covered with trichomes. *Tepals* usually 6, sometimes only 4, rarely 3 or 5, yellow entirely or at least on upper side; *outer tepals* ovate, lanceolate or oblong, acute, with an adaxial subapical appendage, often keeled, covered with trichomes abaxially; *inner tepals* ovate or lanceolate, acute, obtuse, entire or micropapillate at apex, with trichomes along midrib abaxially; *stamens* biseriate, as many as tepals, equal or unequal with outer whorl longer than inner; *filaments* usually subulate, exceptionally filiform or deltoid; *ovary* inferior, trilocular with axile placentation, usually obconical, covered with indumentum. *Capsule* with persisting perianth, transversely circumscissile in upper part and sometimes dehiscing by longitudinal slits as well; *seeds* ovoid or subglobose, with one large papillate appendage, black or brownish; seed coat usually ± papillate.

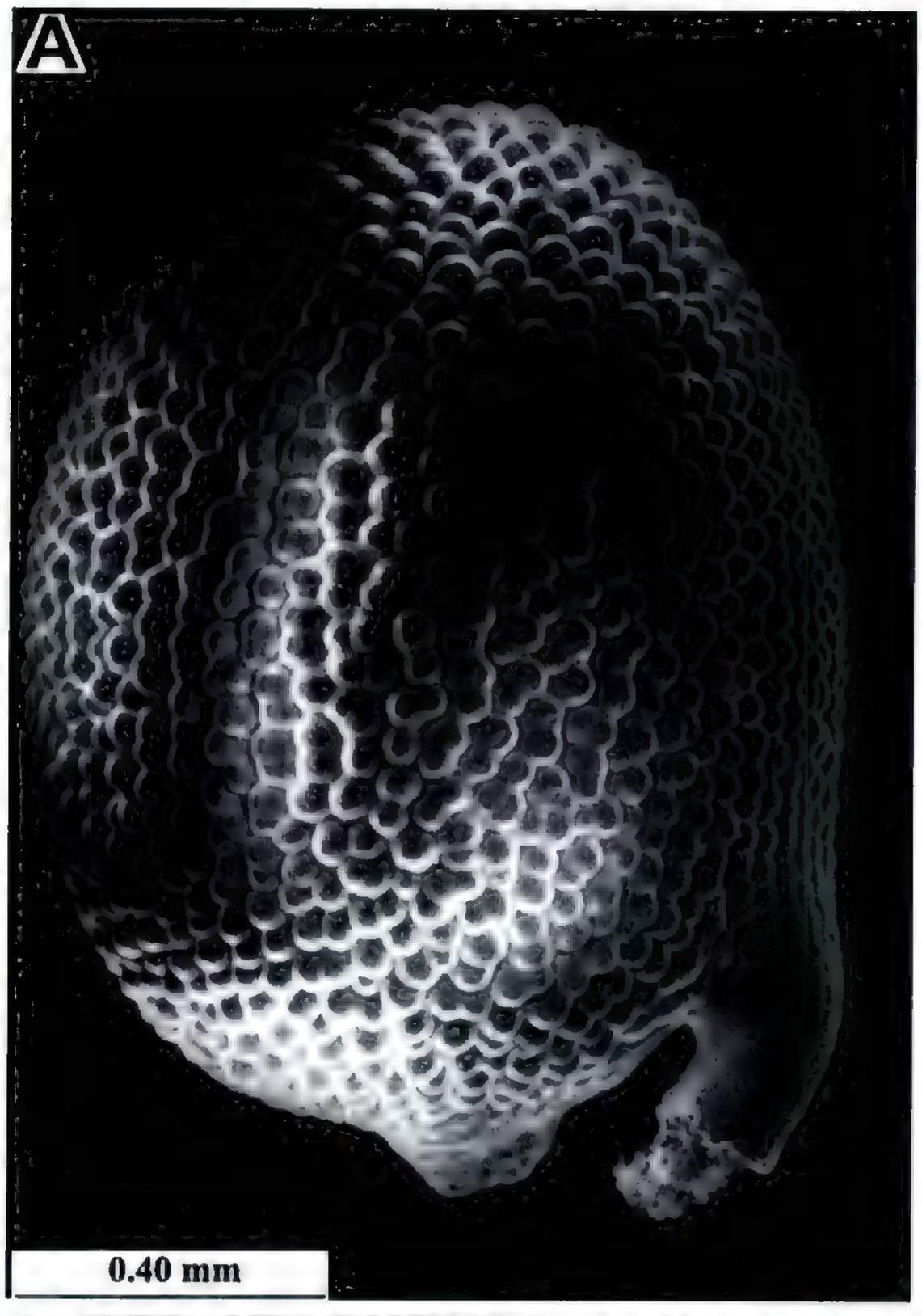
KEY TO *HYPOXIS* SPECIES IN CENTRAL AFRICA

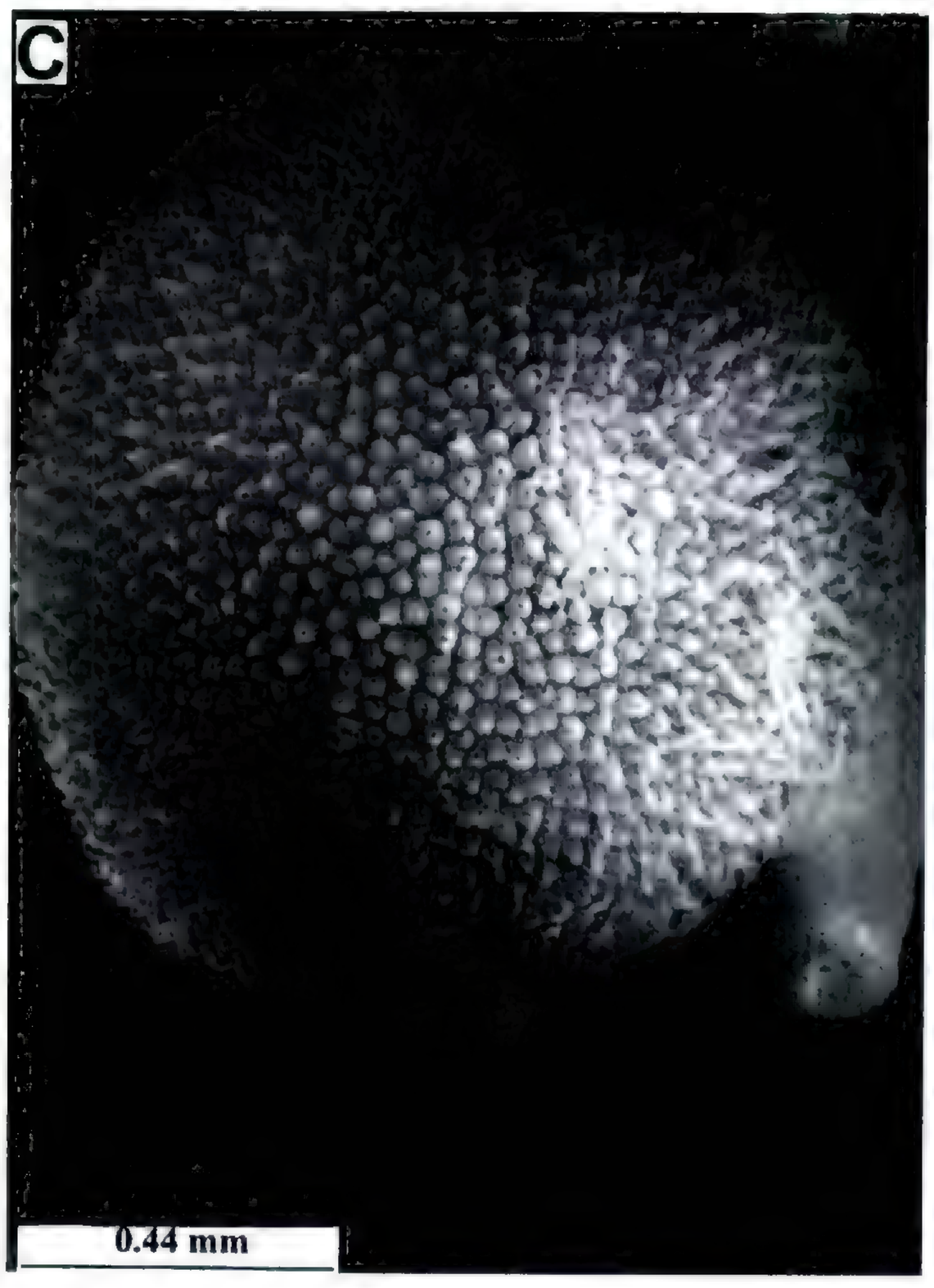
1a. Inner leaves 8–30 mm wide, coriaceous; rhizomes 27–60 mm diam., scapes 2–5 mm wide.
2a. Inner leaves glabrous above, ciliate only on margins and midrib below or entirely covered with indumentum below.
3a. Leaf blade entirely covered with indumentum below.
4a. Flowering sequence acropetal; indumentum red-brown, inner leaves 30–70 (–76) mm wide, (95–) 103–190-nerved ..... 3. *H. bampsiana*
4b. Flowering sequence basipetal; indumentum white; inner leaves 8–20 mm wide, ca. 30-nerved ..... 17. *H. subspicata*
3b. Leaf blade ciliate only on margins and midrib below.
5a. Flowering sequence basipetal, trichomes golden; inner leaves (8–) 20–50 (–70) mm wide ..... 7. *H. goetzei*
5b. Flowering sequence acropetal, trichomes white or gray; inner leaves 6–16 mm wide ..... 1. *H. angolensis*
2b. Inner leaves entirely covered with indumentum on both sides.
6a. Inflorescence 2- to 7-flowered; plant from Haut Zaire Province or Rwanda ..... 20. *H. urceolata*
6b. Inflorescence 6- to 13-flowered; plant from Shaba Province.
7a. Inflorescences accompanied by inner leaves during anthesis ..... 8. *H. hockii*
7b. Inflorescences not accompanied by inner leaves during anthesis ..... 16. *H. robusta*
1b. Inner leaves 1–8 mm wide, usually thin in texture; rhizomes 6–45 mm diam., scapes 0.3–2 mm wide.
8a. Tepals 14–20 mm long ..... 11. *H. lusalensis*
8b. Tepals 6–13 mm long.
9a. Leaves canaliculate, to ca. 1 mm wide.
10a. Tunic membranous, with only few delicate fibers; leaves ca. 0.7 mm wide ..... 6. *H. filiformis*
10b. Tunic fibrous and stiff, leaves ca. 1 mm wide ..... 4. *H. canaliculata*
9b. Leaves carinate or flat, 1 mm or more wide.
11a. Inner leaves ensiform, 6–8 mm wide; tunic fibrous ..... 5. *H. dinteri*
11b. Inner leaves linear, 1–8 mm wide; tunic fibrous or membranous.
12a. Tunic conspicuous, stiff and prominently fibrous or composed of 2–3 mm wide old leaf blades.
13a. Tunic composed of 2–3 mm wide blades often covered with indumentum ..... 10. *H. lejolyana*
13b. Tunic fibrous, glabrous.
14a. Plants 7.5–10 cm high, seeds brown ..... 18. *H. symoensiana*
14b. Plants 20–50 cm high, seeds black ..... 13. *H. malosana*
12b. Tunic not conspicuous, at least partially membranous.
15a. Outer tepals ca. 5 mm wide ..... 19. *H. upembensis*
15b. Outer tepals 2–4 mm wide.
16a. Seeds black.
17a. Scapes bending after anthesis; pedicels 2.5–6 mm long; seed testa colliculate ..... 9. *H. kilimanjarica*
17b. Scapes erect after anthesis; pedicels 7–12 mm long; seed testa honeycombed ..... 12. *H. malaissei*
16b. Seeds brown.
18a. Flowers 1 or 2.
19a. Pedicels 0–5 mm long; scapes bending after anthesis ..... 14. *H. monanthos*
19b. Pedicels 12 mm or more long; scapes erect after anthesis ..... 2. *H. angustifolia*
18b. Flowers 3–6.
20a. Tunic partially fibrous; robust plant known only from Plateau de Muhila ..... 15. *H. muhilensis*
20b. Tunic membranous; slender common plant ..... 2. *H. angustifolia*

1. Hypoxis angolensis Baker, Trans. Linn. Soc. London, Bot. 1: 266. 1878. TYPE: Angola. Huilla: "in collinis dumetosis prope Lopollo, 3800–5500 ft.," 1860, *Welwitsch 4059* (holotype, BM!). Figures 1, 2A, B.

Herb to 48 cm high; *rhizome* ovoid, 6.4–11.0 × 3.9–5 cm (dried out); tunic to 8 cm, usually large and fibrous, brown-red. *Outer leaves* 3 to 5, ovate, 6–14 × 0.8–1.4 cm, ciliate on the margins and midrib below, glabrous above; trichomes tufted, white; nerves of unequal size, (29 to) 39 to 67; *inner leaves* 8 to 27, linear, (12–) 21–48 (–66) × 0.6–1.6 cm wide, in basal part wider and with membranous margins, prominently keeled, ciliate on the margins and midrib below, glabrous above; trichomes tufted, ca. 4-branched with branches 0.5–2.7 mm long, white or gray; thin nerves of equal size, approximate, (29 to) 33 to 67. *Scapes* 3 to 13, 13–27 cm

Figure 1. *Hypoxis angolensis*. —A. Habit. —B. Bract from the basal part of inflorescence in side view. —C. Stamen (ventral view). —D. Style with stigma. —E. Tufted trichome from a scape. —F. Tufted trichome from a leaf edge. A from *Duvigneaud 1321* (BRLU); B from *Plancke 167/2550* (BRLU); C, D, E, and F from *Lisowski, Malaisse & Symoens 13279* (POZG).





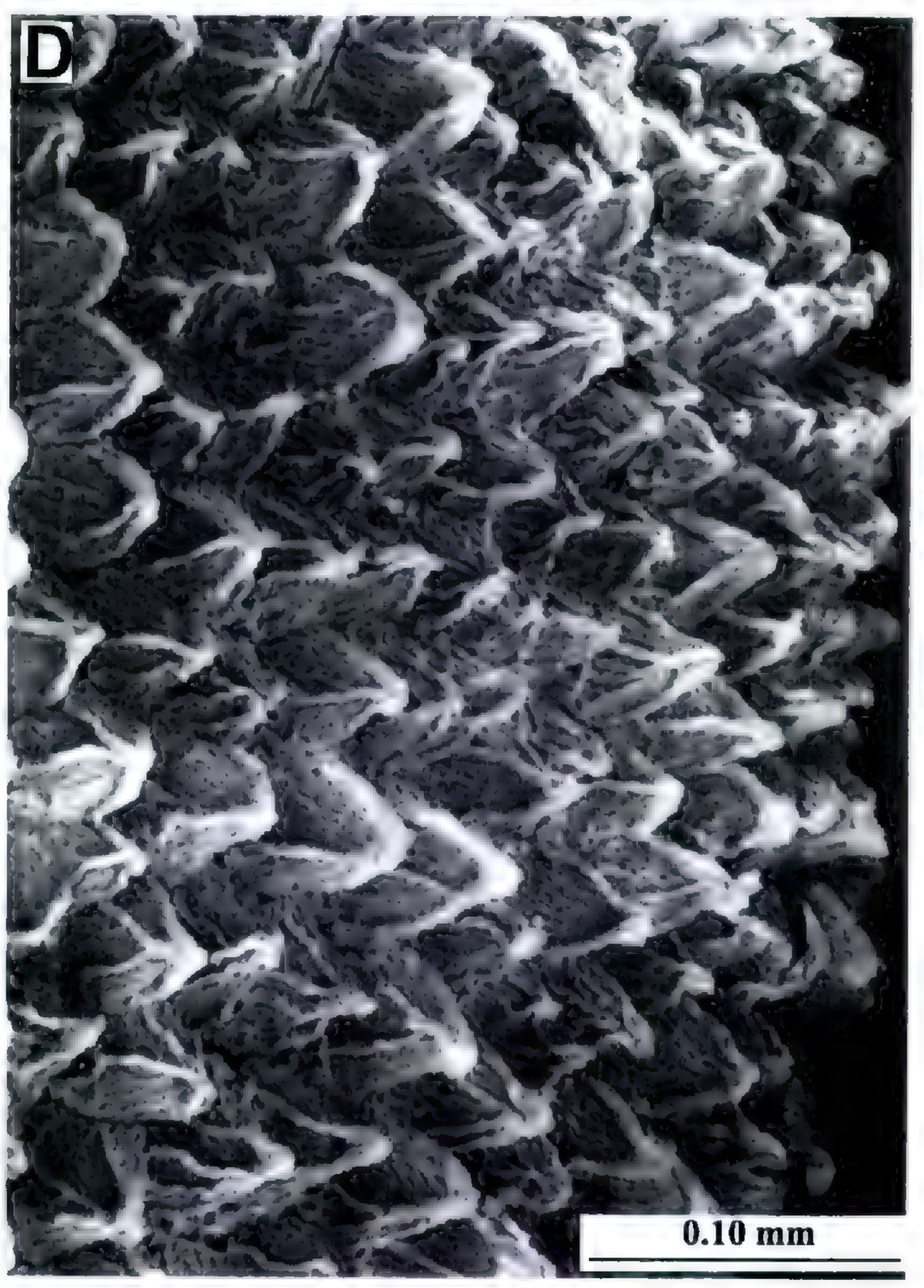


Figure 2. A, B. *Hypoxis angolensis*. —A. Seed. —B. Seed coat sculpture. C & D. *Hypoxis muhilensis* subsp. *kansimbensis*. —C. Seed. —D. Seed coat sculpture. A and B from *Duvigneaud & Timperman 2514* (BR); C and D from *Lisowski 81125* (POZG).

× 3–4 (–5) mm, basally tapering, shortly winged and ciliate, in upper half prominently hispid with tufted trichomes; *raceme* 6- to 12-flowered, floral anthesis acropetal; *bracts* subulate, lowermost 11–25 × 1.5–2.0 mm, 7-nerved, pubescent on midrib and nerves abaxially; upper bracts much smaller, almost filiform; *pedicels* 4–23 mm long, hispid. *Tepals* 6; *outer tepals* oval, 9–16 × 3–5 mm, 5- to 9-nerved with irregular nerves, abaxially hispid; appendage clavate; *inner tepals* ovate, obtuse, 9–14 × 4.5–10.0 mm, 5- to 7 (–10)-nerved with irregular nerves, pubescent abaxially along the midrib; *stamens* subequal or inner shorter than outer; outer stamens 5–9 mm long with filaments 3.5–4.5 mm long; inner stamens 4.5–7.5 mm long with filaments 2.5–3.5 mm long; *anthers* linear, prominently sagittate, slightly emarginate or fused at apex, 3.5–5.0 mm long; *ovary* 4.5–13.0 × 3.5–6.0 mm, hispid; *style* 1–2 mm long; *stigma* pyramidal, 1.5–3.0 mm long. *Capsule* obconical, 6–10 × 4–5 mm, hispid; *seeds* 6 to 9, ovoid, 1.2–1.5 × ca. 1 mm, black; seed coat colliculate.

Distribution and ecology. Congo-Kinshasa (Fig. 22), Angola, Zambia, Tanzania. Wooded grassland with shrubs or trees, often with *Diplorhynchus* and *Hymenocardia*; miombo. Red, degraded, moist, sandy soils; alt. 1340–1800 m. Flowering from June to December. Inner leaves are produced during flowering.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** 60 km N de Sandoa, *Duvigneaud & Timperman 2481* (BRLU); 70 km S de Sandoa, *Duvigneaud & Timperman 2514* (BRLU); 10°48′10″S, 25°16′49″E, *Schaijes 2985* (BR); entre Kansenia et Kapiri, près du village Kamalenge, *Lukuesa 116* (BR); Plateau des Biano, entre Dilungu Yulu et Kansenia, à l'W de la route Tenke-Kansenia, *Symoens 5886* (BR); same plateau, au N de Tenke, *Duvigneaud 1321H* (BRLU); Plateau de la Manika, *Duvigneaud s.n.* (BRLU); environ de Katema, *Lisowski, Malaisse & Symoens 13279* (POZG); près de Djoni, *Lisowski, Malaisse & Symoens 5637* (POZG); Kolwezi, *Plancke 167/2550* (BRLU), *Duvigneaud 4527 H* (BRLU), *Duvigneaud & Timperman 2264* (BRLU), *Schmitz 2977* (BR). ZAMBIA. **Nyanga:** Rochdale Valei, 14.12.1976, *A. Nicholas 502* (MO). TANZANIA. **Iringa:** Iringa–Mbeya Road, vicinity of Ngwazi Estate, *Spjut & Muchai 3462* (MO).

Hypoxis angolensis is a very beautiful species with hispid, light indumentum on leaves and inflorescences (Fig. 1A). Trichomes are usually composed of only four branches, which tend to twist themselves (Fig. 1E, F). The rhizome is often topped with a rich fibrous chestnut-colored tunic. The geographic distribution of *H. angolensis* is very similar to that of *H. subspicata.* Both species share leaves with approximate nerves, but leaves of *H. subspicata* are covered on the whole surface below, whereas leaves of *H. angolensis* are only ciliate on margins and midrib below. These two species also have very different inflorescences. *Hypoxis angolensis* possesses a raceme with acropetal anthesis (Fig. 1A), while *H. subspicata* bears cymes with basipetal anthesis. Moreover, seeds of *H. angolensis* are black with a thin cuticle, whereas seeds of *H. subspicata* are brown with a thick cuticle. *Hypoxis angolensis* is also similar to *H. hockii,* but the latter has leaves totally covered with indumentum and their nerves are visibly distant one from another, not close together like in the former species. Seeds of both species are black, but their seed coat sculptures are different (see Figs. 2A, B and 10C–F).

2. Hypoxis angustifolia Lam., Encycl. 3: 182. 1789. TYPE: Mauritius. *Commerson s.n.* (holotype, P photo!). Figures 3, 18H, I.

Hypoxis luzuloides Robyns & Tournay, Bull. Jard. Bot. État. 25: 254. 1955. TYPE: Congo-Kinshasa. Kivu: "Tschambi, plaine près du pont de la Rwindi," alt. ± 975 m, Oct. 1933, *G. de Witte 1130* (holotype, BR!).

Herb to 10–53 cm high; *rhizome* globose, 0.8–1.2 cm diam. (dried out), white inside; tunic membranous, sometimes with some thin fibers. *Outer leaves* not always present, if present not numerous, grouped in pseudostem, linear in upper part, 10 cm × ca. 7 mm, pilose along midrib and margins below; trichomes 2-branched, white; nerves of unequal size, 5 to 13; *inner leaves* 3 to 12, linear, grass-like, usually keeled, 10–50 cm × 3–8 mm, ciliate on margins and midrib below or very sparsely pilose on entire surface; trichomes 2 (3)-branched, 1.3–2.5 mm long, golden or white, soft; nerves of unequal size 5 to 13 (23), two lateral nerves prominently larger than other. *Scapes* 1 to 6, 5–20 cm × 1 mm, ciliate in lower half, pilose in upper half with 2- to 3-branched trichomes; *flowers* single or in a lax 2- to 6-flowered corymbiform cyme; *bracts* subulate, 12–17 mm long, basally (0.5–) 1.0–1.7 mm wide, 1- or 3-nerved, villous on midrib abaxially, sometimes ciliate on margins; *pedicels* 1.2–2.5 cm long, pubescent. *Tepals* 6 (exceptionally 4), yellow, bright yellow, or outer tepals green and inner yellow, sometimes with a red stripe along midrib; *outer tepals* ovate, 5–8 × 2–3 mm, 5- to 7-nerved with irregular nerves, villous abaxially; appendage clavate; *inner tepals* ovate, obtuse, sometimes minutely papillate on apex, 4–7 × 3–4 mm, 5- to 7-nerved with irregular nerves, pilose along midrib abaxially to 3/4 of its length; *stamens* usually unequal; outer stamens 3–4 mm long with filaments 2.5–3.0 mm long; inner stamens 2–3 mm long with filaments 1.5–2.0 mm long; *anthers* linear,

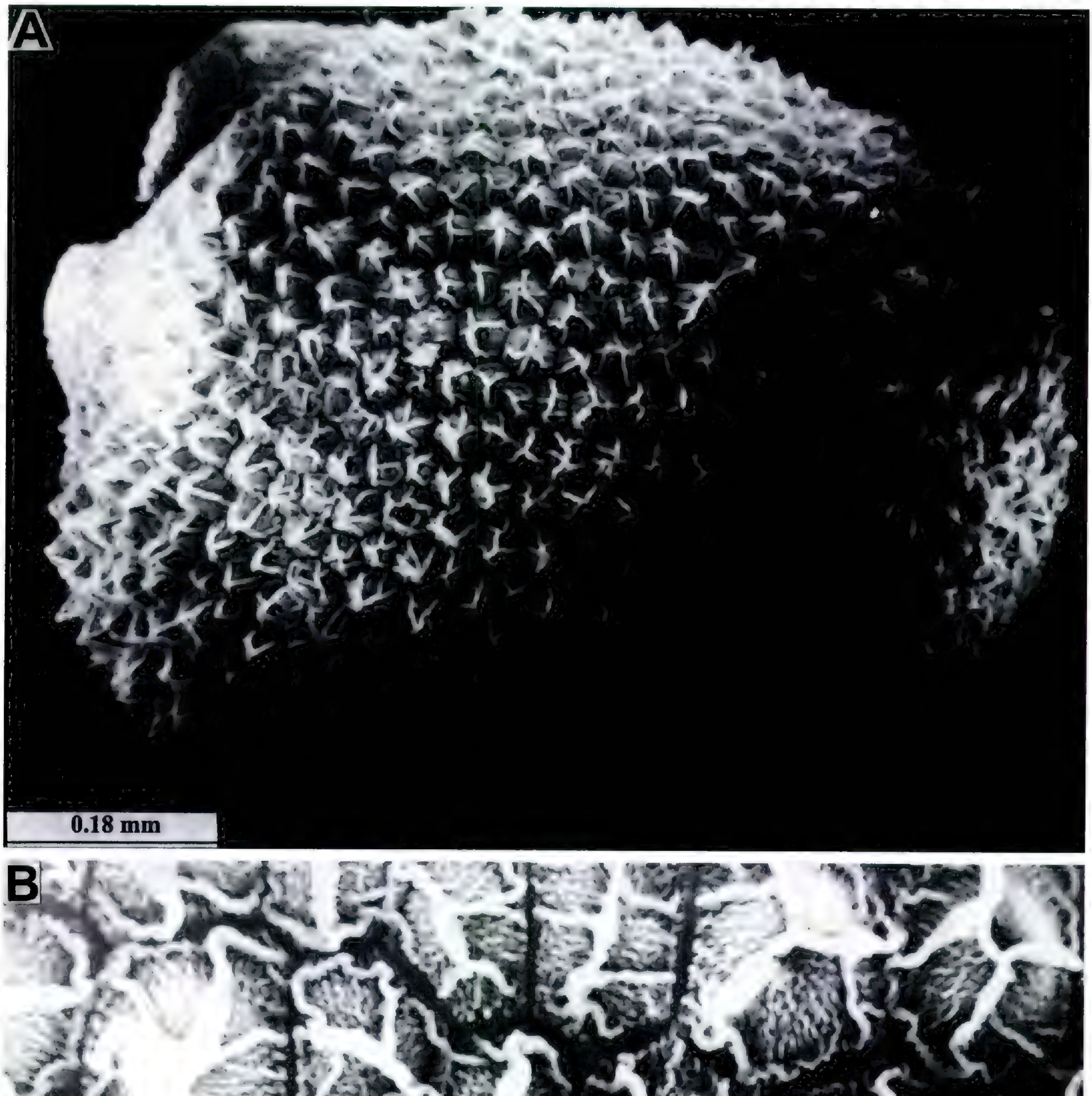


Figure 3. *Hypoxis angustifolia.* —A. Seed. —B. Seed coat sculpture. Both from *Bamps & Malaisse 8052* (BR).

deeply sagittate, retuse at apex, 1.5–3.0 mm long; *ovary* obconical, 2–5 × 1–3 mm, villous; *style* 1–3 mm long; *stigma* 0.7–2.5 mm long, both variable in shape. *Capsule* turbinate, 7–14 × ca. 3 mm, pubescent, often splitting in three lobes; *seeds* numerous, ovoid, ca. 2 mm long; cuticle thick, brown; seed coat with pyramidal pointed projections.

Distribution and ecology. *Hypoxis angustifolia* is a species with a wide geographical range in intertropical and South Africa and from Madagascar and the Mascarene Islands. It occurs both in undisturbed natural habitats like miombo, dry forests, bush, various types of grasslands, river valleys, lakeshores, and in habitats strongly impacted by human activities like pastures, fallow fields, cultivated stands of manioc, and roadsides. It can be found on different kinds of soils: loamy, with kaolin, rocky, laterite, and on granite slabs; alt. 850–1500 (exceptionally 1900) m. Flowering from January to May and in August. It is the only species of *Hypoxis* in Congo-Kinshasa to occur in the western part of the country (Fig. 21).

Additional specimens examined. CONGO-KINSHASA. **Bas-Zaïre:** s. l., *Odon in Gillet s.n.* (BR); Luki, *Wagemans 285* (BR); Bingila, *Dupuis s.n.* (BR); Lutete, *Hens 221* (BR); Mbanza-Ngungu, *Breyne 2624* (BR), *Lisowski 56805* (BR); Kitobola, *Flamigni 297* (BR); Kinkonka, *Vanderyst s.n.* (BR); Kimpasa, *Vanderyst s.n.* (BR); Kisantu, *Gillet s.n.* (BR), *Gillet s.n.* (BR), *Vanderyst 37242* (BR); Pemba, *Williams 18* (BR). **Kinshasa:** environ de Kinshasa, vallée de la Lukaya, *Lisowski 86119* (BR), *Breyne 4711* (BR); Kinshasa, *Pauwels 6009* (BR); Kimuenza, *Lejoly 82/1013* (BR, BRLU); Lutendele, *Jans 98* (BR). **Bandundu:** Chutes Tembo, *Breyne 2590* (BR). **Haut-Zaïre:** entre Arumbi et Berunga, *Louis 4587* (BR); entre Niangara et Wamba, *Lebrun 3256* (BR); Abok, *Scops 168* (BR); route Gabu–Golu, *Taton 437* (BR); Nioka, *Lejoly 3338* (BRLU), *Lisowski 48484* (POZG); Parc National Garamba, ca. piste centrale vers 30 km, *Noirfalise 826* (BR), *Troupin 1308* (BR); piste frontière vers km 15, *Troupin 1243* (BR). **Kasai Oriental:** Lomami, *Dandoy 206 & 361* (BRLU). **Kivu:** Kikanga, *Bequaert 4211* (BR); Katanda, *Lebrun 7557* (BR); Kabare, *Bequaert 5505* (BR); Beni, *Bequaert 3391* (BR); Parc National Virunga, Migeri, *Fredericq in de Witte 8741* (BR); Section Vasongora Ouest, au confluent Ihumbia/Semliki, *de Wilde 273* (BR); Section Vasongora, en bordure routé Beni–Kasindi, *de Wilde 467* (BR); Rwindi, *de Witte 13341* (BR); Kombukabakoli, rive droite Semliki, *de Witte 11267* (BR); entre Kingi et Nyafuma, *Lebrun 7835* (BR); Kibati, *Stauffer 500* (BR); Keshero, *Crispiels-Thonon 132* (BR). **Shaba:** Tshibonde, *Mullenders 2346* (BR); Kapolowe, *Lisowski B-7276 & B-7378* (POZG); Lubumbashi, *Bamps & Malaisse 8052* (BR), *Quarré 5056* (BR); Lusinga, *Lisowski, Malaisse & Symoens 10710A* (POZG); Kumanua, *Bamps & Malaisse 8278* (BR), *Malaisse 8988* (POZG); valleé de Kapiri, *Homblé 1091* (BR); Plateau des Marungu, Pepa, *Lisowski, Malaisse & Symoens 9557 l* (POZG); Parc National Upemba, *de Witte 7508* (BR). RWANDA. Parc National de L'Akagera, *Bouxin & Radoux 434* (BR); environ Gabiro, *Troupin 14109* (BR); Gabiro, *Burgeon 17 & 34* (BR); Kibugabuga, *Liben 620 & 1231* (BR), *Van Oosten 120* (BR); Nemba, *Bouxin & Radoux 1415* (BR); Bugesera, *Augier 2898* (BR); Matinza, *Bouxin & Radoux 1008* (BR); Lulama, *Bouxin & Radoux 418* (BR); Mimuli, *Troupin 4230* (BR); Lukionji, *Troupin 6776* (BR); Kakitumba, *Christiaensen 736* (BR). BURUNDI. Route Bubanza–Musigati, *Lewalle 4189* (BR); Bujumbura, *Lewalle 5486* (BR, MO); plaine de la Ruzizi, *Germain 6086 & 6237* (BR), *Reekmans 2906* (BR, MO); Bulamata, *Germain 7191* (BR); Rumonge, *Lewalle 5029* (BR).

Vernacular name. Nyabokela (dialect Kinyaruanda); Dioko di ngumbi (a partridge tuber, dialect not stated, from Gillet & Pâque, 1910).

The name *H. angustifolia* was used for some time in Central Africa as the name for all species with leaves less than 7 mm wide (Geerinck, 1971). This species is indeed characterized with a great morphological variability, especially in shapes of style and stigma. It differs from all species of *Hypoxis* in an occasional red striping on the tepals, not observed in any other species. In tropical Africa its seeds are always brown and covered with thick cuticle (Fig. 3). Its membranous tunic and seeds distinguish it from *H. malosana,* a species with fibrous tunic and black seeds (Fig. 16). *Hypoxis dinteri,* which bears seeds similar to *H. angustifolia* (Fig. 7), differs in its fibrous tunic and ensiform leaves (Fig. 6C), while leaves of the latter species are linear. *Hypoxis kilimanjarica* possesses black seeds (Fig. 12A) and pedicels not exceeding 6 mm (Fig. 11A), while those of *H. angustifolia* are usually at least 12 mm long. More robust, but similar to *H. angustifolia, H. malaissei* bears a partially fibrous tunic and black honeycombed seeds (Fig. 14B). *Hypoxis monanthos* differs in its partially fibrous tunic and bending scapes with pedicels to 5 mm long (Fig. 11C). Scapes of *H. angustifolia* are rigid. *Hypoxis muhilensis* is a species similar to *H. angustifolia* in its loose inflorescence, sparsely pilose linear leaves, and brown seeds, which have a similar sculpture in *H. muhilensis* subsp. *kansimbensis* (Fig. 2C), but quite different in the typical subspecies (Fig. 17). Moreover, *H. muhilensis* possesses a fibrous tunic and is a much more robust plant. *Hypoxis upembensis* shares similar seeds (Fig. 20C, D) and indumentum with *H. angustifolia* (Fig. 18H, I), but is easy to distinguish thanks to its partially fibrous tunic, 5 mm wide outer tepals, and pedicels to 6 mm long (Fig. 6E). Tepals of *H. angustifolia* do not exceed 4 mm in width.

Hypoxis angustifolia is a medicinal plant with a pulp from the rhizome used for healing pustules and infected wounds (Gillet & Pâque, 1910).

Figure 4. Habit of *Hypoxis bampsiana* from *Lisowski 7653* (POZG).

3. Hypoxis bampsiana Wiland, Bull. Jard. Bot. État. 66: 207. 1997. TYPE: Congo-Kinshasa. Shaba: "Plateau des Kundelungu, au bord de la rivière Lofoi," alt. 1500 m, Oct. 1969, *Lisowski, Malaisse & Symoens 7653* (holotype, POZG!). Figures 4, 5A, B.

Herb to 45 cm high; *rhizome* ovoid, ca. 5.5 × 4.5–6.0 cm (dried out); tunic membranous and fibrous, to 8 cm. *Outer leaves* 4 to 7, ovate, cuspidate, keeled and reflexed, (1.3–) 5.0–18.0 × (1–) 3–5 cm, tomentose below, glabrous above; trichomes tufted, golden, longer on margins and midrib; nervation with 65 to 105 unequal nerves; *inner leaves* 6 to 7, ovate or lanceolate, cuspidate, slightly keeled, erect or slightly reflexed, (12–) 17–45 (–56) × 3.0–7.0 (–7.6) cm, tomentose below, glabrous above; trichomes tufted, golden, longer on margins and midrib, midribs tufted, ca. 13-branched with branches varying 0.2–1.6 mm; nervation with 95 to 191 unequal nerves. *Scapes* 6 to 9, 14.5–30 cm × (2–) 3–4 mm, short winged and ciliate in lowest ⅔, wider and tomentose in apical ⅓; trichomes tufted, ca. 9-branched, branches 0.2–3.5 mm long; *raceme* 6- to 14-flowered, floral an-

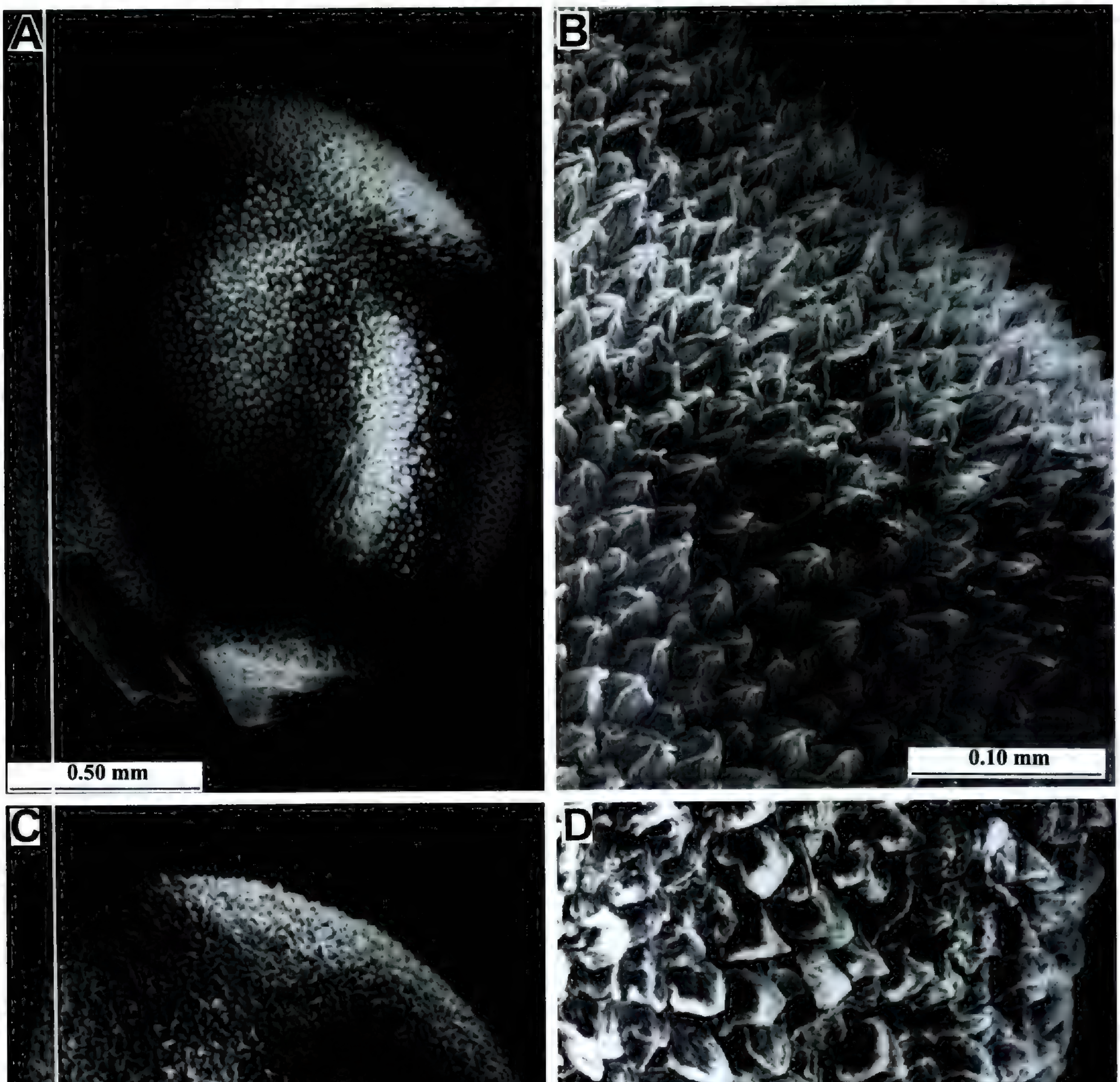


Figure 5. A, B. *Hypoxis bampsiana.* —A. Seed. —B. Seed coat sculpture. C & D. *Hypoxis goetzei.* —C. Seed. —D. Seed coat sculpture. A and B from *Lisowski 1009* (POZG); C and D from *Lisowski 7492* (POZG).

thesis acropetal; *bracts* subulate, acute, keeled, 1–2 cm × 1.5–4.0 mm, lowermost 5-nerved, hairy on midrib and nerves abaxially; *pedicels* 0.4–2.3 cm long, tomentose. *Tepals* 6; *outer tepals* oblong, slightly keeled, 14–15 × 5.5–6.0 mm, abaxially pubescent, ± regularly 9- to 14-nerved; appendage clavate, 1 mm long; *inner tepals* wide ovate, obtuse, with margins inflexed below apex, ca. 13 × 7–8 mm, with ca. 11 irregular nerves, pubescent abaxially along midrib on lowest 1/3; *stamens* equal, ca. 8 mm long; *filaments* ca. 5 mm long; *anthers* linear, prominently sagittate, emarginate at apex, 5–6 mm long; *ovary* obconical, pubescent, 6–10 × 3–5 mm; *style* trigonous, 1–3 mm long; *stigma* pyramidal, obtuse, ca. 2 mm long, of three linear papillate surfaces. *Capsule* turbinate, 4–10 × 4–8 mm, pubescent; *seeds* numerous, ovoid, 1 mm diam.; cuticle thick, brown; seed coat bristly with pointed pyramidal projections winged with cuticle.

Distribution and ecology. Congo-Kinshasa (Fig. 22), Zambia. Montane grassland, wooded grassland, woodland; miombo; on sandy soils ; alt. 1250–1650 m. Flowering January and October. Inflorescences are produced simultaneously or after inner leaves.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Plateau des Kundelungu, *Lisowski, Malaisse & Symoens 737 & 7615* (POZG); entre les rivières Petite Lofoi et Kalembe, *Lisowski, Malaisse & Symoens 12947 & B-7279* (POZG); au bord de la rivière Kalembe, *Lisowski, Malaisse & Symoens 12830, 12853 & 12859* (POZG); près du gîte RACK, *Lisowski, Malaisse & Symoens 1009* (POZG).

Hypoxis bampsiana is closely related to sympatric *H. goetzei.* Both species possess ovate leaves, very similar brown seeds (Fig. 5), and golden tufted trichomes (Fig. 8H–J), but of a different distribution on the leaves. Leaves of *H. bampsiana* are covered with indumentum on the whole laminal surface below (Fig. 4), whereas leaves of *H. goetzei* are only ciliate on margins and midrib below. The most distinctive difference is observed in the inflorescence types. Inflorescences of *H. bampsiana* are racemose, with floral anthesis being acropetal, whereas cymes of *H. goetzei* are characterized by basipetal anthesis. This distinguishes these two species even without leaf material, which is an important character, because *H. goetzei* often develops flowers before leaves.

4. **Hypoxis canaliculata** Baker, Trans. Linn. Soc. ser. 2, Bot. 1: 265. 1878. TYPE: Angola. Huilla: "regio subtemperata, in pascuis collinis arenosis prope Lopollo, frequens," *Welwitsch 4057* (holotype, BM!). Figure 6A.

Herb to 9–15 cm high; *rhizome* subglobose 14–24 mm (dried out); tunic to 4–6 cm, fibrous and membranous; roots not very thick. *Outer leaves* ca. 2, canaliculate, ca. 4 cm × 1 mm, villous below; trichomes tufted, white; nerves 7, unequal; *inner leaves* ca. 3, canaliculate, 4.5–15.0 cm × 1 mm, prominently villous on margins and midrib below; trichomes tufted, white, with branches falling off, so on old leaves only simple and 2-branched trichomes occur; nerves 7, unequal. *Scapes* 2 to 8, 2.5–5.0 cm × 0.5 mm, villous with white tufted trichomes; *flowers* single or two; *bracts* subulate, acute, 3 × 0.3 mm, villous abaxially; *pedicels* to 8 mm long, villous. *Tepals* 6; *outer tepals* lanceolate, ca. 10 × 2 mm, villous abaxially with tufted trichomes, 7-nerved; *inner tepals* lanceolate, acute, 9 × 1.5 mm, villous along midrib abaxially, irregularly 5-nerved; *stamens* unequal; outer stamens ca. 5 mm long with filaments ca. 3 mm long; inner stamens ca. 3 mm long with filaments ca. 1 mm long; *anthers* linear, sagittate, 3 mm long, fused at apex; *ovary* obconical, 3–4 × ca. 2.5 mm wide, villous; *style* tapering toward apex, ca. 2 mm long; *stigma* pyramidal, acute at apex, composed of three stripes of papillae, 2.5 mm long. *Capsule* subglobose; *seeds* ovoid, black; seed coat almost smooth, glittering, dotted, and with small papillae near apex.

Distribution and ecology. Congo-Kinshasa (Fig. 27), Angola, Zambia, Malawi, Zimbabwe. Grassland areas ; alt. 1350 m. Flowering in November (in Angola, December to January, *Baker 1878a*).

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Domain de Muhila, près du village Baton, *Lisowski 81123* (POZG). ZAMBIA. Mbala [= Abercorn]. *Fries 1275* (UPS).

Hypoxis canaliculata was originally described from Angola and subsequently collected in Zambia (Fries, 1916; Richards & Morony, 1969), Zimbabwe (Norlindh & Weimarck, 1937), and Malawi (Binns, 1968), but never before has it been reported from Central Africa. *Hypoxis canaliculata* shares its canaliculate leaves with *H. filiformis,* but has 1 mm wide leaves and a fibrous tunic (Fig. 6A). Leaves of *H. filiformis* are 0.7 mm wide, filiform, and the tunic is membranous with only a few soft fibers (Fig. 6D). *Hypoxis canaliculata* is similar in appearance to *H. symoensiana* (Fig. 6B), but differs in black seeds with a thin cuticle, which are not brown and covered with a thick cuticle as in the latter species (Fig. 20A). Because few collections are known, more studies will be needed to determine its taxonomic position and relationships to other species.

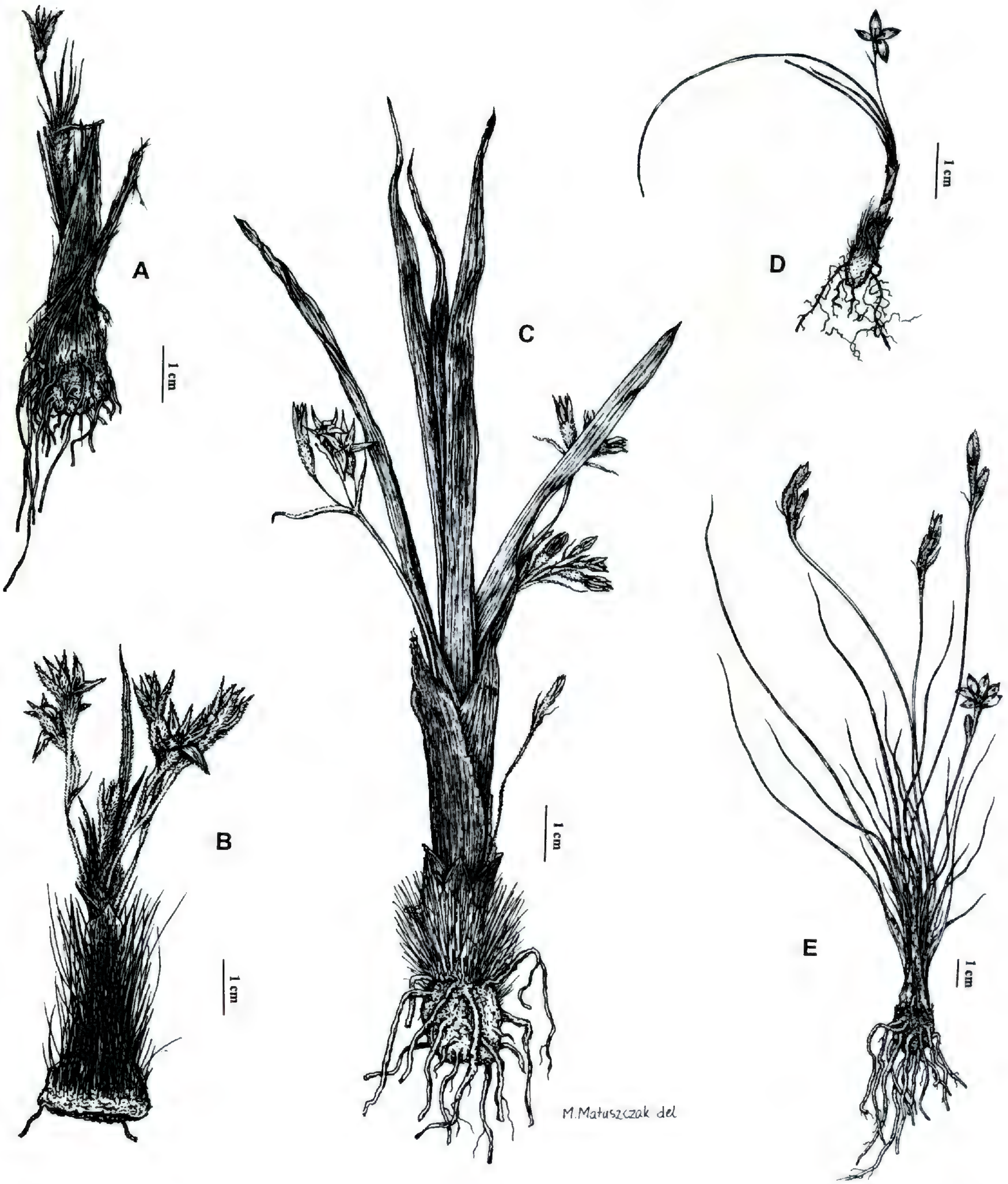
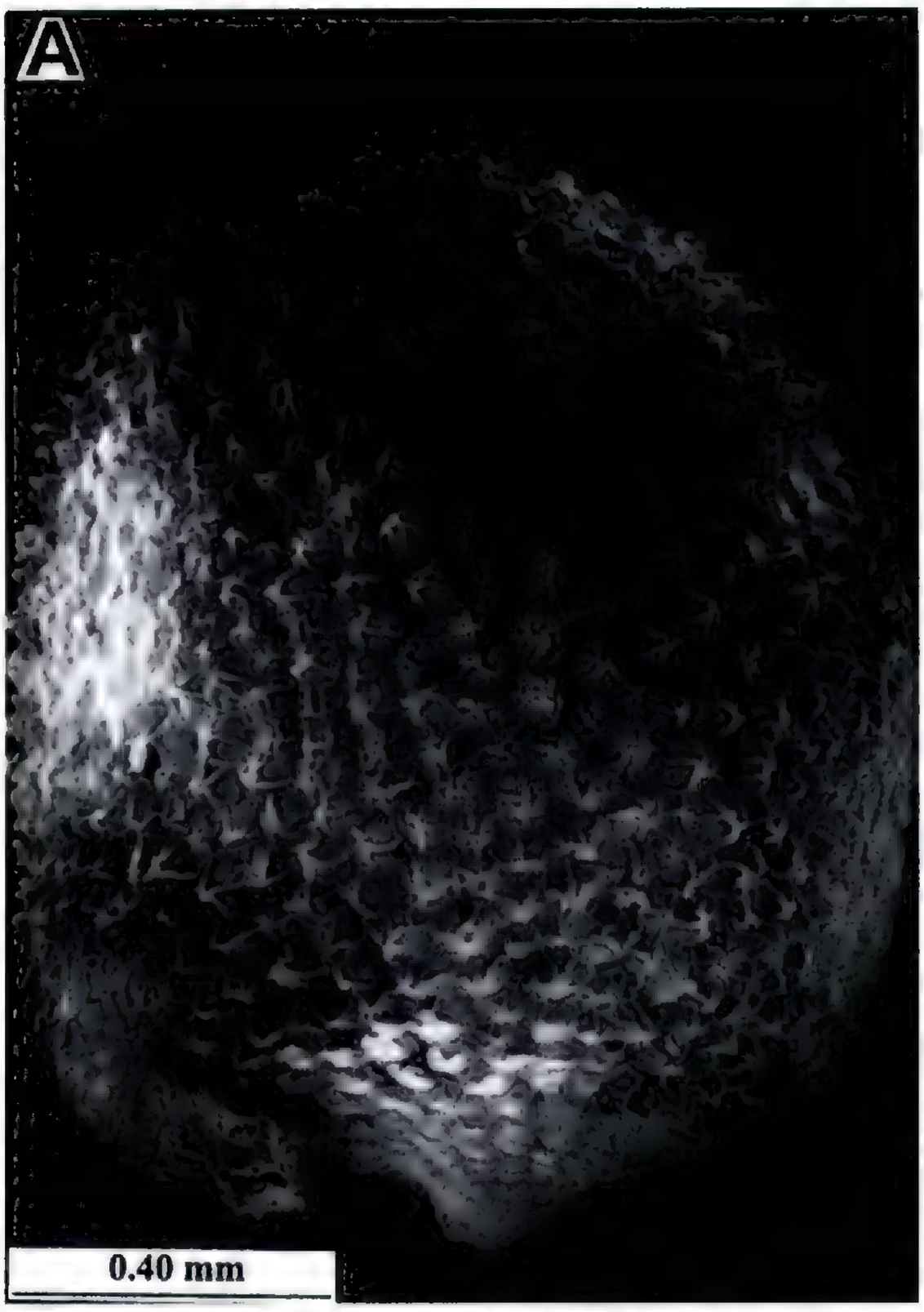
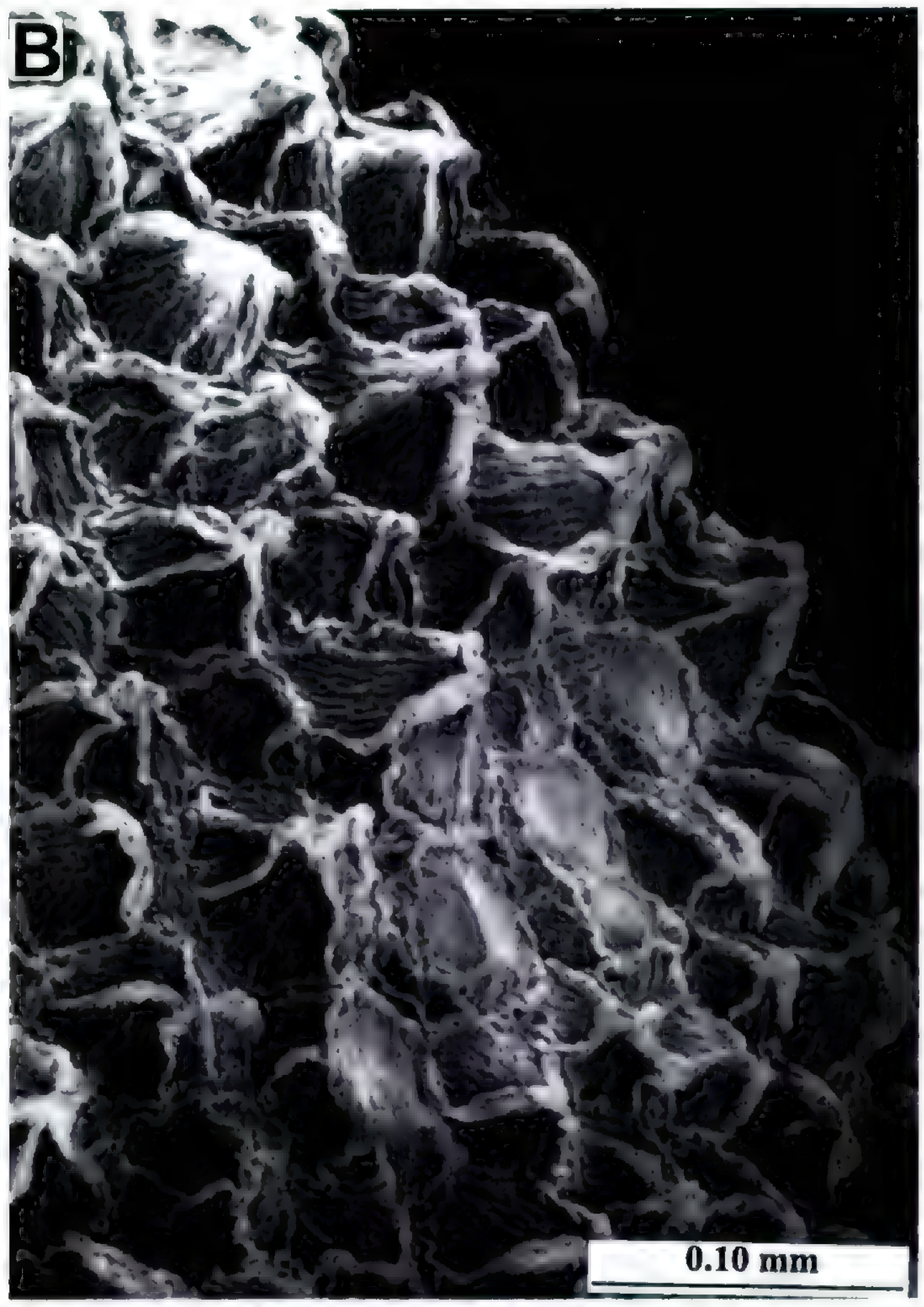


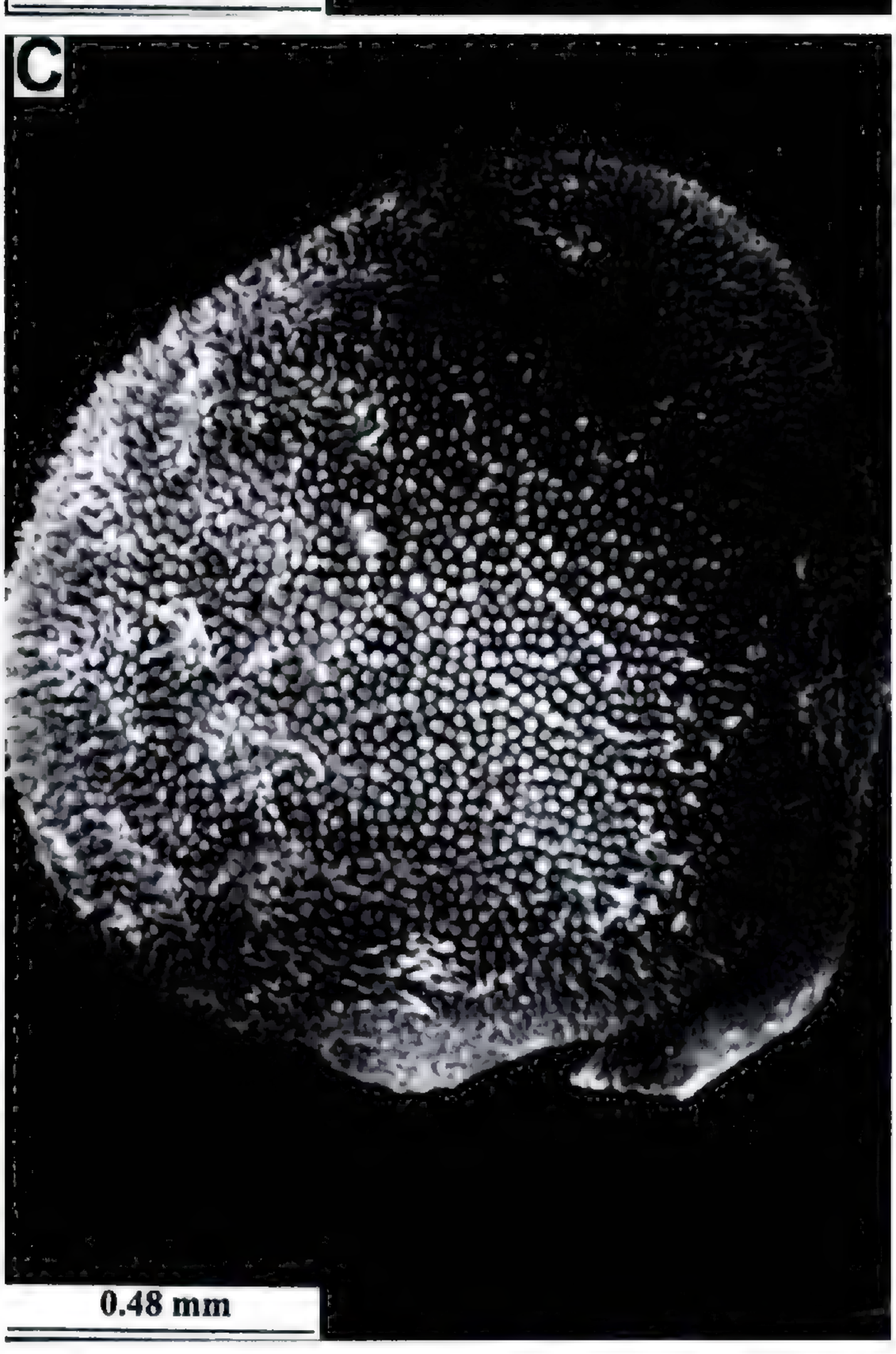
Figure 6. Habit. —A. *Hypoxis canaliculata* (*Lisowski 81123*, POZG). —B. *Hypoxis symoensiana* (*Lisowski, Malaisse & Symoens 12058*, POZG). —C. *Hypoxis dinteri* (*Bulaimu 58*, BR). —D. *Hypoxis filiformis* (*Niyongere 95*, BR). —E. *Hypoxis upembensis* (*de Witte 3698*, BR).

5. Hypoxis dinteri Nel, Bot. Jahrb. Syst. 51: 302. 1914. TYPE: Namibia. Damaraland: Otavital, Jan. 1909, *Dinter 634* (holotype, B!). Figures 6C, 7A, B.

Herb to 22 cm high; *rhizome* ovoid, 0.7–1.3 cm diam. (dried out); tunic fibrous, 1.5 cm high. *Outer leaves* 2 to 3, ovate, 1.5–11.0 cm × 8–20 mm, ciliate on margins and midrib below; trichomes 2-branched, golden, long; nerves 9 to 19, unequal; *inner leaves* 3 to 5, ensiform, sometimes keeled, 7–22 cm × 6–8 mm, long hispid on margins and midrib below and pilose on entire surface with golden 2-branched trichomes; nerves 21 to 25, unequal. *Scapes* 2 to 4, 4.5–10.5 cm × 1 mm wide, narrowly winged at base, above ciliate, pubescent apically;







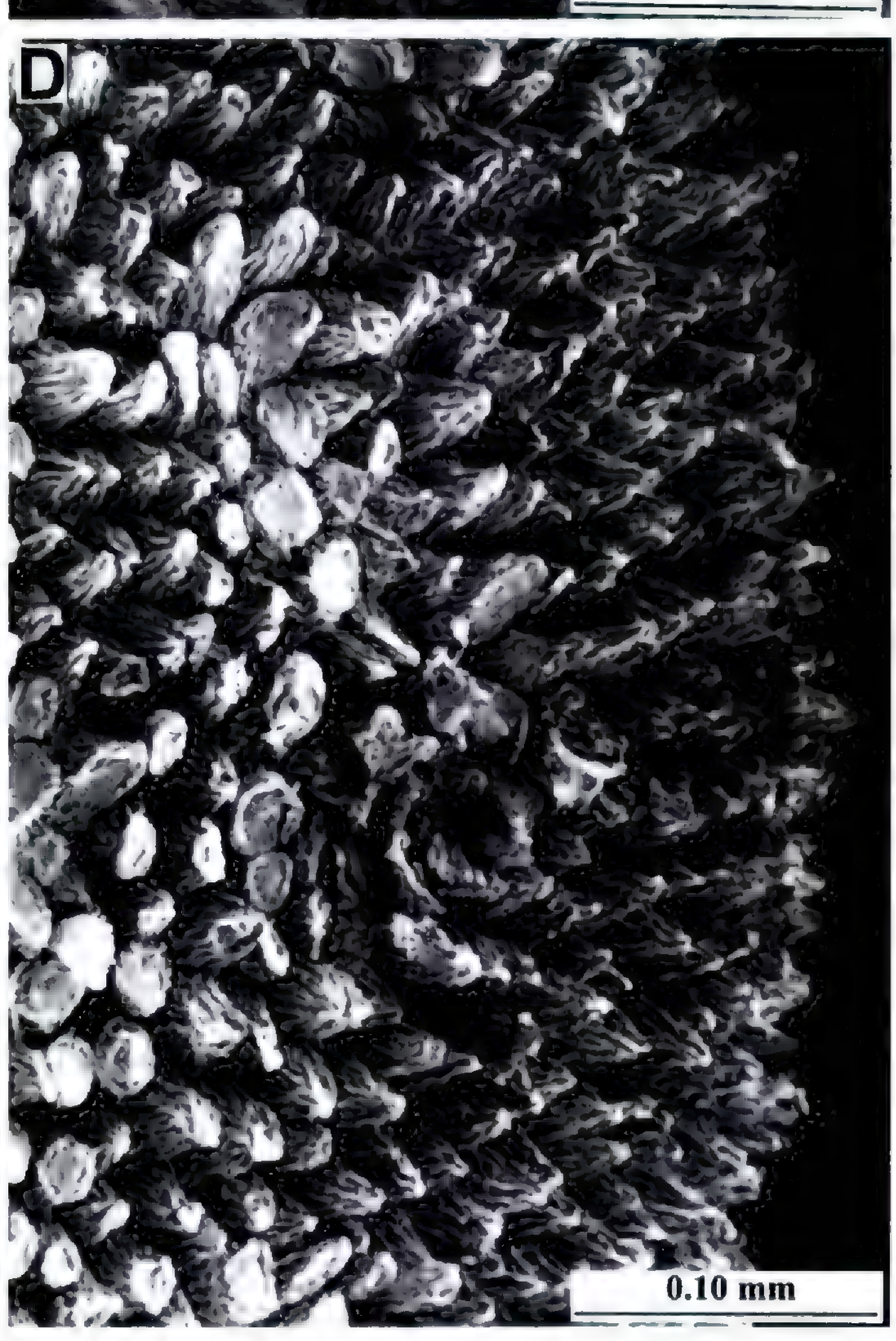


Figure 7. A, B. *Hypoxis dinteri.* —A. Seed. —B. Seed coat sculpture. C & D. *Hypoxis lusalensis.* —C. Seed. —D. Seed coat sculpture. A and B from *Detilleux 229* (BR); C and D from *Lisowski, Malaisse & Symoens 8300* (POZG).

cyme corymbiform, 2- to 4-flowered; *bracts* subulate 4.5–15.0 × 1 mm, pubescent along nerves abaxially with 2-branched trichomes, ciliate in apical part with simple trichomes, the lowermost larger than the upper; *pedicels* 6–14 mm long, pubescent. *Tepals* 6; *outer tepals* elliptic, 5–7 × 2–3 mm, pubescent abaxially; nerves 5 to 7, irregular; *inner tepals* ovate, acute, 5–7 × ca. 3 mm, pubescent along midrib adaxially; nerves 5 to 7, irregular; *stamens* equal, 3–4 mm long; *filaments* 2.0–2.5 mm long; *anthers* oblong, 1.7–2.0 mm long, sagittate, retuse at apex; *ovary* obconical, 3–5 × ca. 2–3 mm, pubescent; *style* trigonous, 2–3 mm long; *stigma* pyramidal with three strips of papillae, obtuse at apex, 1–2 mm long. *Capsule* obovoid, 7–8 × 2.5–3.0 mm, pubescent; *seeds* numerous, ovoid, ca. 1.3 × 1 mm; cuticle thick, dark red-brown; seed coat bristly with pointed projections.

Distribution and ecology. Congo-Kinshasa (Fig. 27), Zambia, Namibia. Occurs in miombo; natural pasturage, dembo; on gritty yellow soil; alt. ca. 1250 m. Flowering from November to December.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** A 11 km au NW de Lubumbashi, *Bulaimu 58* (BR); Keyberg, *Detilleux 229* (BR); Mokambo–Tela, km 10, riv. Tshinshimuka, *D'Hose 48* (BR). ZAMBIA. **Kitwe:** 7 mi. N of Kitwe, 1275 m, 14.11.161, *K. Linley 212* (MO).

Hypoxis dinteri was until now only known from the type location in Namibia. The plants on the holotype sheet are single-flowered, possessing one or two bracts. Two bracts are often noted on single flowers in other species. For example, *H. kilimanjarica* and *H. monanthos* may bear two or one flower with two bracts due to degeneration of one bud. In contrast, specimens from Congo-Kinshasa have 2 to 4 flowers (Fig. 6C). Quite unique ensiform leaves (Fig. 6C) entirely covered with 2-branched golden trichomes and a fibrous tunic indicate, however, that this is the same species. A corymbiform cyme and brown seeds of *H. dinteri* (Fig. 7A) make this species similar to *H. angustifolia* (Fig. 3), but the latter species bears a membranous tunic and linear leaves.

6. Hypoxis filiformis Baker, J. Linn. Soc., Bot. 17: 109. 1878b. TYPE: South Africa. Queenstown, 1860, *Cooper 462* (holotype, K!; isotype, B!). Figure 6D.

Herb to 10 cm high; *rhizome* cylindrical, 1.2 × 0.7 cm (dried out); tunic black, membranous with some light-colored, soft fibers, to 1.5 cm long. *Outer leaves* 2, ca. 6 mm wide, cuspidate, much shorter than inner leaves, glabrate; *inner leaves* 3 to 4, basally subulate, above filiform and canaliculate, 3.2–16.0 cm × ca. 0.7 mm wide, erect or bending backward, 3-nerved, glabrate with a few 2- to 3-branched light-colored trichomes on nerves in basal part of lamina below. *Scapes* 1 to 4, to ca. 5 cm × ca. 0.3 mm, basally wider, widely winged, ciliate, pubescent in the upper half with 2- to 3-branched trichomes; *flowers* solitary; *bracts* subulate, ca. 4 × 1 mm at base, pubescent on the midrib abaxially; *pedicels* 3.0–4.5 mm long, pubescent. *Tepals* 6 or 4; *outer tepals* elliptic, ca. 6 × 2 mm, pubescent abaxially especially along the midrib with 2- to 3-branched trichomes, 5-nerved; *inner tepals* elliptic, obtuse, ca. 5 × 2 mm, pubescent abaxially along midrib, 5-nerved; *stamens* 4, unequal; outer stamens ca. 3 mm long with filaments ca. 2.5 mm long; inner stamens ca. 2.5 mm long with filaments ca. 1.5 mm long; *filaments* subulate, distinctly tapering apically; *anthers* sagittate, fused at apex, ca. 1 mm long; *ovary* obconical, ovoid after flowering, ca. 3.5 × 1 mm, prominently pubescent; *style* terete, ca. 1 mm long; *stigma* composed of 3 free lobes, less than 1 mm long. *Capsule* and *seeds* not seen.

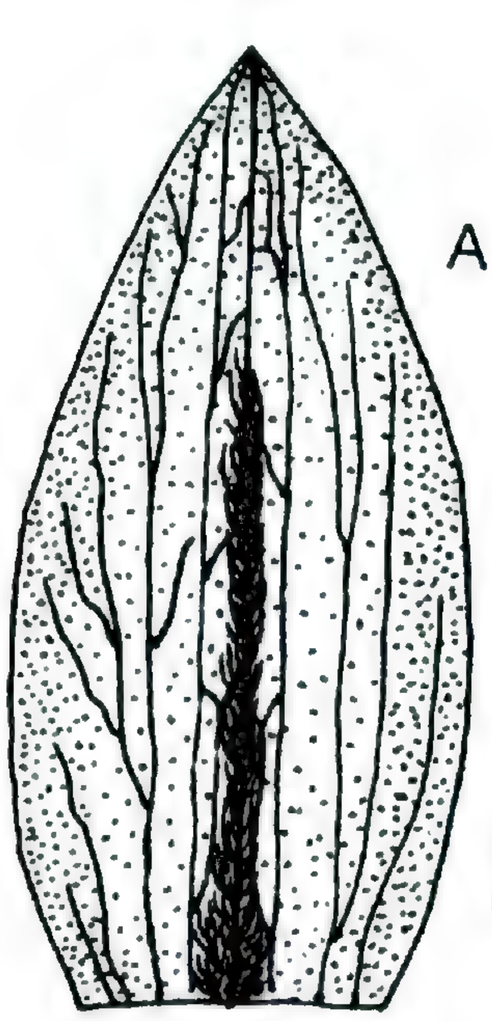
Distribution and ecology. Burundi (Fig. 27), Lesotho, South Africa. Rocky and wet grassland. Flowering in January in Burundi.

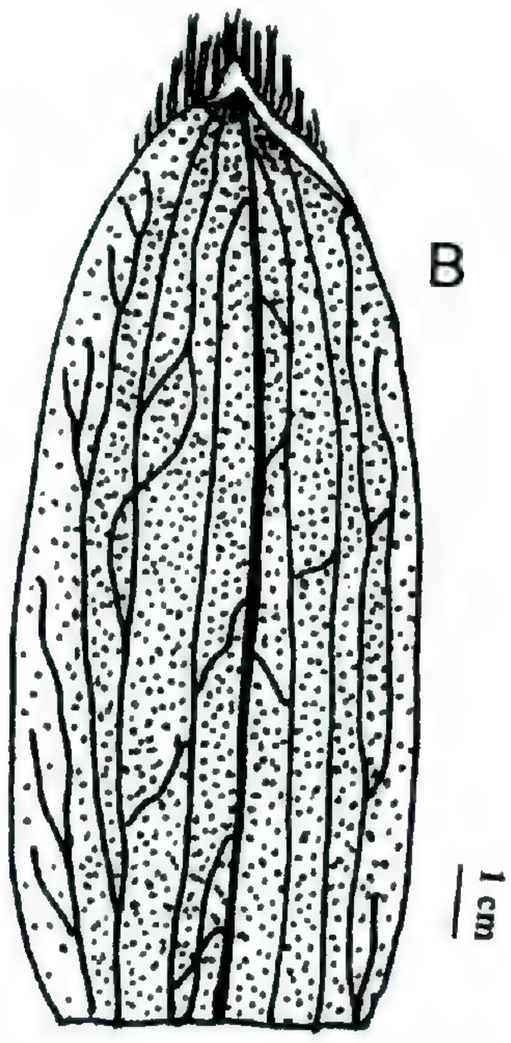
Additional specimen examined. BURUNDI. Territory Mwaro: Nyamiyaga près de Kisozi, alt. 2150 m, *Niyongere 95* (BR).

Hypoxis filiformis was confused in the past with *H. malosana* (De Wildeman, 1921; Zimudzi, 1996). However, these two species differ noticeably. *Hypoxis filiformis* bears a mainly membranous tunic with few soft fibers and filiform, canaliculate inner leaves only 0.7 mm wide. *Hypoxis malosana* possesses a prominent fibrous black tunic and flat or carinate leaves 1–3 mm wide.

Hilliard and Burtt (1983) described *H. tetramera,* which differs from *H. filiformis* in possessing only 4 tepals and retuse anthers. Such retuse anthers were described as well in *H. sagittata* Nel (1914b), who also mentioned that some flowers have 4 tepals. The Central African plant bears 4-tepaled flowers as well (Fig. 6D), but with fused anthers. The single character of fused or retuse anther apices does not appear to be a satisfactory basis for separating these three species, but this problem will have to be studied in South Africa, where they all are sympatric. So far, for the Burundi plant I decided to use the oldest name.

In Central Africa *H. filiformis* is most similar to *H. kilimanjarica* subsp. *kilimanjarica.* Some plants of the latter bear carinate leaves about 1 mm wide, so they look very similar to canaliculate leaves of

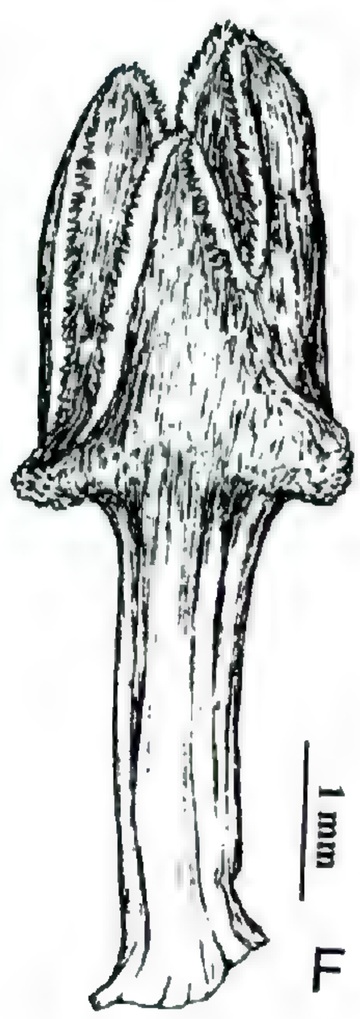

A
1 cm


B
1 cm

C
2 mm

1 mm
D

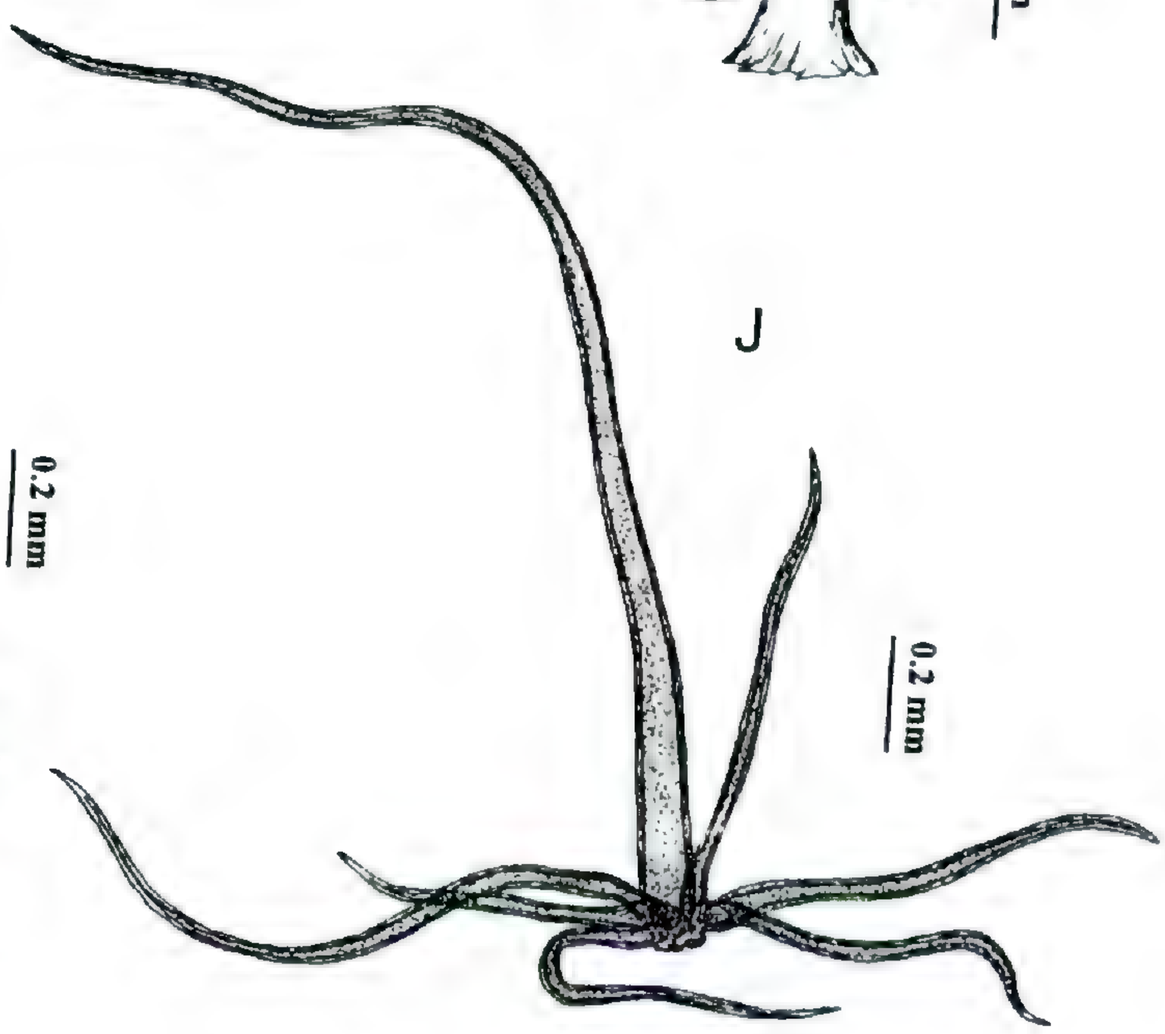
E
1 mm


1 mm
F

G
1 mm

H
0.2 mm

I
0.2 mm


J
0.2 mm

the former. Moreover, flowers of *H. kilimanjarica* are sometimes 4-tepaled. Usually, however, inner leaves of *H. kilimanjarica* are at least 2 mm wide with 9–13 nerves (Fig. 11A), while inner leaves of *H. filiformis* are only 0.7 mm wide and 3-nerved.

7. **Hypoxis goetzei** Harms, Bot. Jahrb. Syst. 30: 276. 1901. TYPE: Tanzania. "Unyika, bei Dorf Toola, um 1300 m ü. M.," 8 Nov. 1899, *Goetze 1416* (holotype, B!; isotypes, BM!, BR!). Figures 5C, D, 8, 13C.

Hypoxis rubiginosa Nel, Bot. Jahrb. Syst. 51: 320. 1914. TYPE: Mozambique. "Unteres Mgaka-Tal, um 1200 m ü M.," Jan. 1901, *Busse 947* (holotype, B!).

Hypoxis esculenta De Wild., Repert. Spec. Nov. Regni. Veg. 11: 537. 1913. TYPE: Congo-Kinshasa. Ober-Katanga, 1911, *Hock s.n.* (holotype, BR!).

Herb to 41 cm high; *rhizome* subglobose, 3.2–5.7 cm diam. (dried out); tunic fibrous, black, to 9 cm. *Outer leaves* 3 to 7, ovate, cuspidate, older slightly keeled and reflexed, 5.0–12.2 (–15.0) × 2–5 cm, ciliate on the margins and midrib below, glabrous above; trichomes tufted, brown or dark golden; nerves unequal, 31 to 69; *inner leaves* 6 to 8, long elliptic or sword-shaped, acute, entire or sometimes with very small teeth, erect, sometimes keeled, (7.5–) 11.0–30.5 (–41) × (0.8–) 2.0–5.0 (–7.0) cm, ciliate on margins and midrib below, glabrous above; trichomes tufted, ca. 5-branched, brown or dark golden; nerves unequal, 27 to 91. *Scapes* 1 to 8, 8.0–25.5 cm × 2.5–5.0 mm, ciliate in lowest ⅓, prominently tomentose in apical ⅔; trichomes tufted, ca. 7-branched with branches 0.6–2.3 mm long, getting darker and falling off with age; *cyme* 4- to 14-flowered, floral anthesis basipetal; *bracts* widely subulate, keeled, (5–) 7–17 × 1–4 mm, the lowermost 5- to 7-nerved, hairy on the midrib abaxially, ciliate on margins in apical part; *pedicels* 6–10 mm long, on young flowers in the basal part of inflorescence almost none. *Tepals* 6; *outer tepals* ovate or lanceolate, keeled, 10.5–18.0 × 4–6 (–7) mm, abaxially pubescent; nerves irregular 5 to 9; inner tepals ovate, acute, with margins inflexed below apex, 10–17 × (4–) 5–8 (–9) mm; pubescent abaxially along the midrib on lower ⅔; nerves irregular, (5) 7 to 9; *stamens* equal, 6–10 mm long; *filaments* subulate, 3.0–4.5 mm long; *anthers* prominently sagittate, slightly emarginate or fused at apex, 4–7 (–8) mm long; *ovary* obconical, (3–) 6–7 (–9) × 3–6 (–7) mm, pubescent; *style* often tapering toward base, 2.5–4.0 mm long; *stigma* (1.2–) 2.0–3.0 (–4.0) mm long, composed of three free lobes variously covered with papillae. *Capsule* turbinate, 4–10 × (3–) 4–6 mm, pubescent; *seeds* ca. 12, globose or almost ovoid, 1.2–1.5 × 1.2–1.5 mm, brown; cuticle thick; seed coat bristly with pointed pyramidal projections winged with cuticle.

Distribution and ecology. Congo-Kinshasa (Fig. 26), Kenya, Tanzania, Zambia, Zimbabwe, Mozambique. Grassland and wooded grassland, on plateaus or in river valleys, near springs; in miombo with *Brachystegia boehmii* Taub. On dry to swampy ferralit, eolith, sometimes very compact soils; alt. 1200–1830 m. Flowering from August to November.

Additional specimens examined. CONGO-KINSHASA. **Kivu:** source de la rivière Kipese, *Plancke 63/911* (BRLU). **Shaba:** 20 km SO d'Lubumbashi, *Schmitz 4803* (BR); Luiswishi, *Malaisse 13696* (BR); 28 km au NE de la Lubumbashi, *Bulaimu 715* (BR); Kasonta, *Schmitz 7499* (BR); route entre Mitwaba et Kiubo, *Duvigneaud & Timperman 2731* (BR); Mitwaba, *Duvigneaud & Timperman 2700* (BR), *Mortelmans 89* (BR); Katuba, *Quarré 704* (BR, MO), *921* (BR); Dilolo, 10 km au N de la fôret de la plateau à *Marquesia, Duvigneaud & Timperman 2400* (BRLU); Kansenia, *de Witte 4* (BR); Lubudi, *Quarré 3416* (BR); vallée de la Dikuluwe, *Hock s. n.* (BR); route Kabiasha–Kasomeno, près du village Bowa, *Lisowski 50994* (POZG); vallée de la Luapula, près de Kiniama, *Lisowski B-7270* (POZG); Plateau des Marungu, Mulongoshi, *Quarré 7252* (BR); 7 km au NW de Pepa, *Lisowski, Malaisse & Symoens 8665* (POZG); Kibobwa, 1 km au S de Mukunda, *Lisowski, Malaisse & Symoens 8669* (POZG); Mont Kibobwa, près du village Kibobwa, *Lisowski, Malaisse & Symoens 11798* (POZG); Plateau de Biano, Dilungu Yulu, *Duvigneaud & Timperman 2627* (BRLU); Grelco, *Quarré 6035* (BR); Parc National de L'Upemba, entre Kabwe Kanono et Mukana, *de Witte 4406* (BR); entre Lusinga [= Mitwaba] et Kabwe Kanono, *de Witte 7364* (BR); Lusinga, *de Witte 2714* (K) & *Robyns 3587* (BR); vers tête de source Kalumengongo, *de Witte 7451* (BR); tête de la source de la Mukelengia, *de Witte 7307* (BR); près rivière Dipidi, *de Witte 2809* (BR); Plateau des Kundelungu, *Lisowski, Malaisse & Symoens 7492* (POZG), *Lisowski 81107* (POZG); au bord de la rivière Lofoi, *Lisowski 7655* (POZG); Tilwizembe, *Plancke 127-1741* (BRLU).

Hypoxis goetzei is a useful plant with an edible rhizome. De Wildeman (1913) described this spe-

←

Figure 8. *Hypoxis goetzei.* —A. Dorsal view of inner tepal. —B. Ventral view of outer tepal. —C. Dorsal view of bract. —D & F. Morphological variability of stigmas. —E, G. Stamen (E dorsal view, G ventral view). —H. Tufted trichome from a leaf midrib. —I. Tufted trichome from a leaf edge. —J. Tufted trichome from a scape. A and B from *Lisowski, Malaisse & Symoens 8665* (POZG); C from *Schmitz 7499* (BR); D and E from *Lisowski B-7270* (POZG); F, G, H, I, and J from *Lisowski, Malaisse & Symoens 11798* (POZG).

cies for Congo-Kinshasa as *H. esculenta*. *Hypoxis goetzei* was confused by some authors (De Wildeman, 1914; Geerinck, 1971) with *H. subspicata*. Both species have basipetal floral anthesis but they differ in leaf shape and indumentum both on vegetative and reproductive organs. *Hypoxis goetzei* possesses long elliptic or sword-shaped inner leaves, ciliate only on the margins and midrib below. Whereas *H. subspicata* bears linear leaves entirely covered with indumentum below, indumentum of *H. goetzei* is golden, and that of *H. subspicata* white. Seeds of both species are covered with thick cuticle (Figs. 5C, D, 10 A, B). *Hypoxis goetzei* is probably most closely related to *H. bampsiana*. Both species possess similar seeds (Fig. 5), indumentum of brown or golden rather short tuft hairs (Figs. 8H–J, 13C), and appearance, but leaves of *H. bampsiana* are entirely covered with indumentum below. Cymes of *H. goetzei* are characterized by basipetal anthesis, whereas racemes of *H. bampsiana* are with acropetal floral anthesis. This distinguishes these two species even without leaf material, a character worth noticing, because *H. goetzei* often develops flowers before leaves. Bracts of *H. goetzei* are often ciliate (Fig. 8C), which is uncommon in both other mentioned species.

8. Hypoxis hockii De Wild., Repert. Spec. Nov. Regni. Veg. 11: 537. 1913. TYPE: Congo-Kinshasa, "Ober-Katanga," 1911, *Hock s.n.* (syntypes, B!, BR!).

Hypoxis pedicellata Nel ex De Wildeman, Bot. Jahrb. Syst. 51: 320. 1914. TYPE: Democratic Republic of Congo, "Lualaba," 1891, *Deschamps s.n.* (holotype, BR!).

Herb to 78 cm high; *rhizome* cylindrical or ovoid, 2.7–3.5 cm diam. (dried out); tunic fibrous, stiff, to 25 cm. *Outer leaves* 2 or 3, linear, elliptical or oblong, keeled, 3–20 × 1–5 cm, pubescent on both surfaces or only below; nerves unequal, 17 to 39; *inner leaves* 3 to 15, linear, narrowing toward the apex, keeled, 10.5–78.0 (–102.0) × 0.8–2.0 cm, densely villous on the whole surface or prominently villous on margins and midrib below and only sparsely pubescent on the lamina; nerves unequal, 11 to 39. *Scapes* 3 to 12, 7–34 (–50) cm × 2–4 mm, thickened beneath inflorescence, winged and ciliate at base, villous apically with long white or golden tufted trichomes; *raceme* 6- to 13-flowered, floral anthesis acropetal; *bracts* subulate, keeled, the lowermost 7–22 × 1–2 mm, 1- or 3-nerved, villous on the midrib abaxially; *pedicels* 2–20 mm long, prominently villous. *Tepals* 6, yellow or orange-yellow; *outer tepals* ovate or elliptic, (9–) 10–16 × (3–) 4–6 mm wide, densely hispid abaxially, 5- to 9-nerved; *inner tepals* ovate, shortly cuspidate or obtuse at apex, (8–) 10–15 × (5–) 6–8 mm, abaxially pilose on midrib, 5- to 7 (to 9) -nerved; *stamens* about 5–10 mm long; *filaments* subulate or almost linear, 3.5–4.5 mm long; *anthers* linear, sagittate, fused or retuse at apex, 4–8 mm long; *ovary* obconical, 3–9 × (2–) 3–5 mm, densely pilose; *style* terete or trigonous 1.0–5.0 mm long; *stigma* 1–4 mm long, composed of 3 linear lobes fused to apex or 3 free triangular lobes with papillae on margins. *Capsule* turbinate, (4–) 5–9 × 3–5 mm, pubescent; *seeds* several, ovoid or spherical, 1.5–2.0 × 1.0–1.5 mm, black; seed coat variously papillate.

Distribution and ecology. *Hypoxis hockii* is known only from Congo-Kinshasa (Figs. 25, 27) and Zambia. It occurs in miombo, grasslands, and dry forest, on sandy-loamy soil; alt. 1200–2000 m. Flowering from July to August and from October to November.

Hypoxis hockii was included in the synonymy of *H. subspicata* (Geerinck, 1971). There are, however, features that distinguish these two species quite well. First, flower anthesis of *H. hockii* is acropetal, whereas that of *H. subspicata* is basipetal. Second, inner leaves of *H. hockii* are totally covered with trichomes and their nerves are distinct one from another. Inner leaves of *H. subspicata* have indumentum below only and approximate nerves. Moreover, seeds of the first species are black (Fig. 10C–F) and covered with a thin cuticle, while those of the second are brown and covered with a thick cuticle (Fig. 10A, B). *Hypoxis hockii* is also similar to *H. angolensis*, but inner leaves of the latter species have approximate nerves and are only ciliate. Trichomes on the margins and midrib of leaves and from scapes of *H. hockii* (Fig. 9E, F, I, J) possess usually more branches than trichomes of *H. angolensis* (Fig. 1E, F). Seeds of both species are black, but the seed coat sculpture differs (Figs. 2A, B, 10C–F). Another species that bears resemblance to *H. hockii* is *H. robusta*. These two species differ in seed coat sculpture (Figs. 10C–F, 14C, D) and in the shape of inner tepals, which are obtuse or cuspidate in *H. hockii* and acuminate in *H. robusta*. Moreover, flowers of the former are usually accompanied by the inner leaves, whereas in the latter, flowers always appear long before inner leaves, and only outer leaves can be seen with them, if any persist.

Hypoxis hockii is a species characterized by great variability. Although all plants are rather similar in appearance, their indumentum density, seed coat sculpture, and floral features like stigma shape or

filaments vary greatly (Fig. 9). The seed coat sculpture is its most distinctive feature. Plants of almost equivalent stature can bear very different seeds (Fig. 10C–F). I decided to stress this observed seed heteromophism by designating three varieties. Less dense indumentum of the leaves of *H. hockii* var. *katangensis*, compared with other varieties, is a helpful feature in its delimitation as well. However, because of still insufficient material and lack of field studies, it is not possible to decide how this observed variation is distributed in this species. In my opinion, there are not enough differences to apply a subspecies rank to the delimited groups; still I think that the existing ones are worth varietal emphasis.

1a. Seed coat colliculate; outline of cells clearly visible; cell boundaries channeled; outer periclinal walls flat, micro-papillate ..... 8b. *H. hockii* var. *colliculata*

1b. Seed coat bristly; outline of cells not clearly visible; cell boundaries raised; outer periclinal walls convex with papillae conical and obtuse or nipple-shaped and aculeate.

2a. Papillae conical and obtuse ..... 8a. *H. hockii* var. *hockii*

2b. Papillae nipple-shaped and aculeate ..... 8c. *H. hockii* var. *katangensis*

8a. Hypoxis hockii var. hockii. Figures 9F, 10C, D.

Herb to 30 cm high; *rhizome* ca. 3 cm; tunic to 4.8 cm. *Outer leaves* ca. 2, oblong, 3–12 × 1–3 cm, pubescent, especially on margins, with long, tufted trichomes; nerves ca. 21; *inner leaves* 4 to 6, linear, 12–25 × 0.8–1.4 cm, spathe-like in basal part, densely villous with long, white, tufted trichomes; nerves unequal 15 to 25, 4 or 6 nerves much larger than others. *Scapes* ca. 4, 14–30 cm × 2–4 mm, narrowly winged and ciliate at base, trichomes white; *raceme* 9–11-flowered; *bracts* 3-nerved; *pedicels* 2–14 mm long. *Outer tepals* ovate, 14–15 × ca. 6 mm, 7-nerved; *inner tepals* shortly cuspidate at apex, 13–14 × ca. 7 mm, pilose on lower third of midrib, 5-nerved; *stamens* 5–7 mm long; *filaments* subulate, ca. 3.5 mm long; *anthers* fused at apex, 4–5 mm long; *ovary* 4–9 × ca. 3 mm; *style* terete, 4.5–5.0 mm long; *stigma* composed of 3 linear lobes fused to apex, 1.5–2.0 mm long. *Capsule* 5–9 × 3–4 mm; *seeds* ovoid, ca. 2 × 1.5 mm; seed coat with conical papillae obtuse at apex.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** s.l., *Hock s.n.* (BR); Kaniama, *Quarré 2673* (K) ; Plateau des Kundelungu, *Lisowski, Malaisse & Symoens 7565* (POZG).

Hypoxis hockii var. *hockii* is known only from a few collections, including a type collection of the species. Black seeds with smooth conical papillae (Fig. 10C, D) distinguish this taxon from other varieties.

8b. Hypoxis hockii var. **colliculata** Wiland, var. nov. TYPE: Congo-Kinshasa. Shaba: Plateau des Kundelungu, on the riverside of Lofoi, *Lisowski, Malaisse & Symoens 7682* (holotype, POZG!). Figures 9A–E, 10F.

Haec varietas *H. hockii* var. *hockii* et var. *katangensis* affinis, sed ab eis testa colliculata micropapillataque distinguitur.

Outer leaves linear, 5–20 × (1.0–) 1.6–4.0 cm, pubescent especially below; nerves 17 to 23 (to 31); *inner leaves* 15–78 (–102) × 0.8–2.0 cm, prominently villous on margins and midrib below and sparsely villous on whole surface with bright tufted trichomes; nerves 11 to 31. *Scapes* 3 to 7, 17–34 (–50) cm × ca. 3 mm wide, broadly winged and ciliate in basal part; raceme 6- to 12-flowered; the lowermost *bracts* 10–17 × ca. 2 mm, 3-nerved, bracts in apical part of inflorescence 5–10 × ca. 1 mm wide, abaxially pubescent on midrib; *pedicels* 4–20 mm long, prominently pubescent. *Tepals* 6, yellow or orange-yellow; *outer tepals* elliptic, (9–) 13–16 × (3–) 5–6 mm, 5- to 9-nerved; *inner tepals* ovate, shortly cuspidate at apex, (8–) 11–15 × (5–) 6–8 mm, abaxially pilose on two lower thirds of midrib, 5- to 7 (to 9)-nerved; *stamens* 5–8 mm long; *filaments* subulate, 3.5–4.5 mm long; *anthers* fused or retuse at apex, 4–7 mm long; *ovary* (3–) 4–6 × (2–) 3–5 mm; *style* subterete or trigonous, 1.0–3.5 mm long; *stigma* 1–4 mm long, wider than style or equal, obtuse at apex, composed of 3 linear papillate receptive surfaces or of 3 free triangular lobes with papillae on margins. *Capsule* (4–) 5–7 × 3–5 mm diam.; *seeds* spherical or ovoid, ca. 2 × 1.5 mm, black and matte; seed coat colliculate and micropapillate.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Environs de Lubudi, *Cabu 36* (BR); Nkala, Kambove, *Pauwels 6922* (BR); Kipopo, 25 km ONO d'Elisabethville, *Schmitz 4199* (BR); Luiswishi, *Malaisse 13695* (BR); Katubu, *Quarré 765* (BR); Plateau des Kundelungu, au bord de la rivière Lofoi, *Lisowski, Malaisse & Symoens 7654* (POZG); Plateau des Marungu, Mount Kampilikwe, 3 km au NNW de Kionta, *Lisowski, Malaisse & Symoens 8574a* (POZG).

The rhizome of *Hypoxis hockii* var. *colliculata* is edible. This variety is the most common and variable taxon within *H. hockii* in Central Africa. Polymorphism in stigma and stamen shape may be observed among these plants (Fig. 9A–D). Seeds with

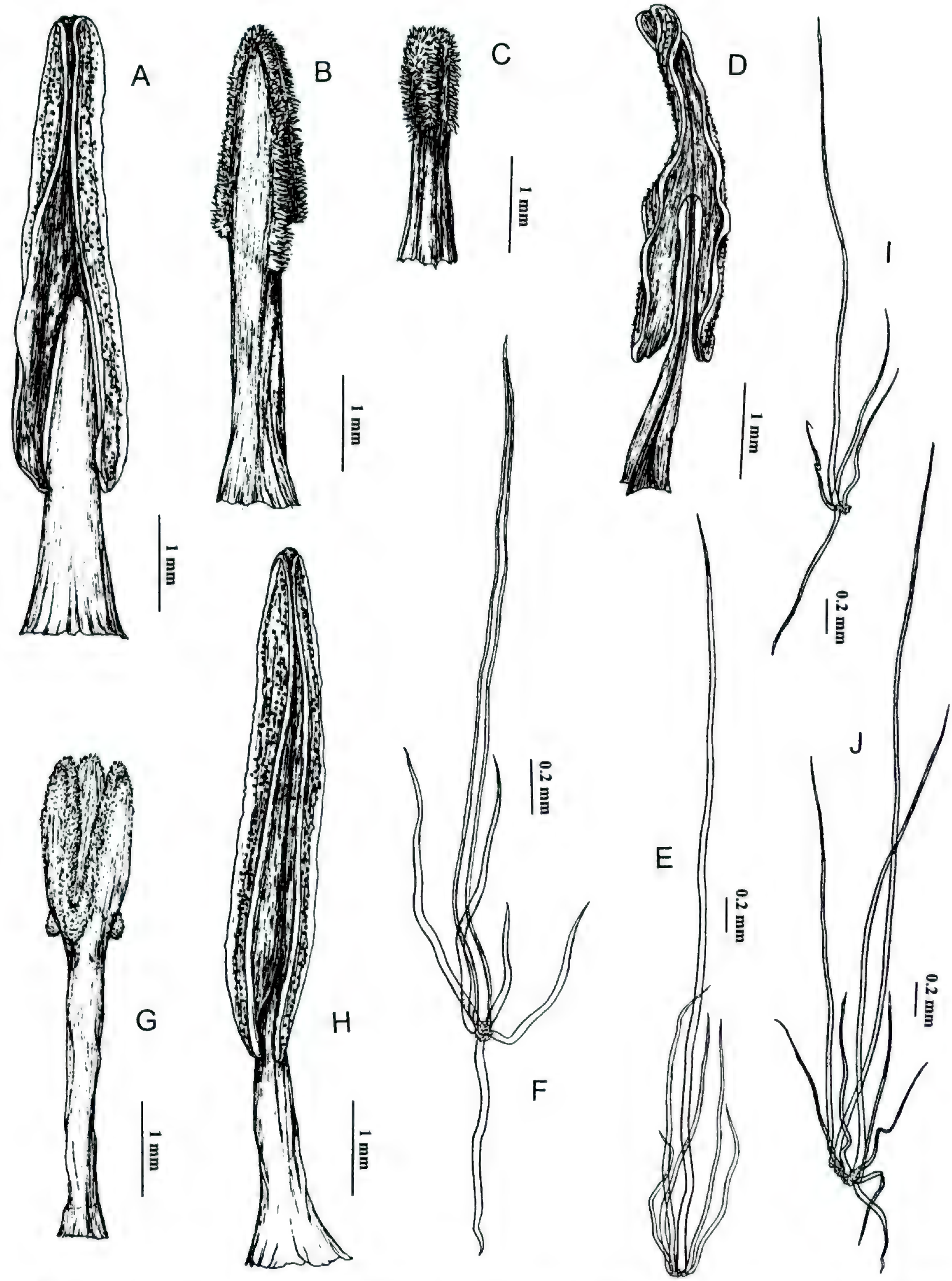


Figure 9. A–E. *Hypoxis hockii* var. *colliculata.* —A. Stamen fused at apex (ventral view). —B. Pyramidal stigma. —C. Oblong stigma. —D. Stamen retuse at apex. —E. Tufted trichome from a scape. —F. *Hypoxis hockii* var. *hockii*, tufted trichome from a scape. G–J. *Hypoxis hockii* var. *katangensis.* —G. Free-lobed stigma. —H. Stamen (ventral view). —I. Tufted trichome from a leaf midrib. —J. Tufted trichome from a leaf margin. A and B from *Lisowski, Malaisse & Symoens 7682* (POZG); C and D from *Schmitz 4199* (BR); E from *Lisowski, Malaisse & Symoens 7654* (POZG); F from *Lisowski, Malaisse & Symoens 7565* (POZG); G–J from *Lisowski, Malaisse & Symoens 11797* (POZG).

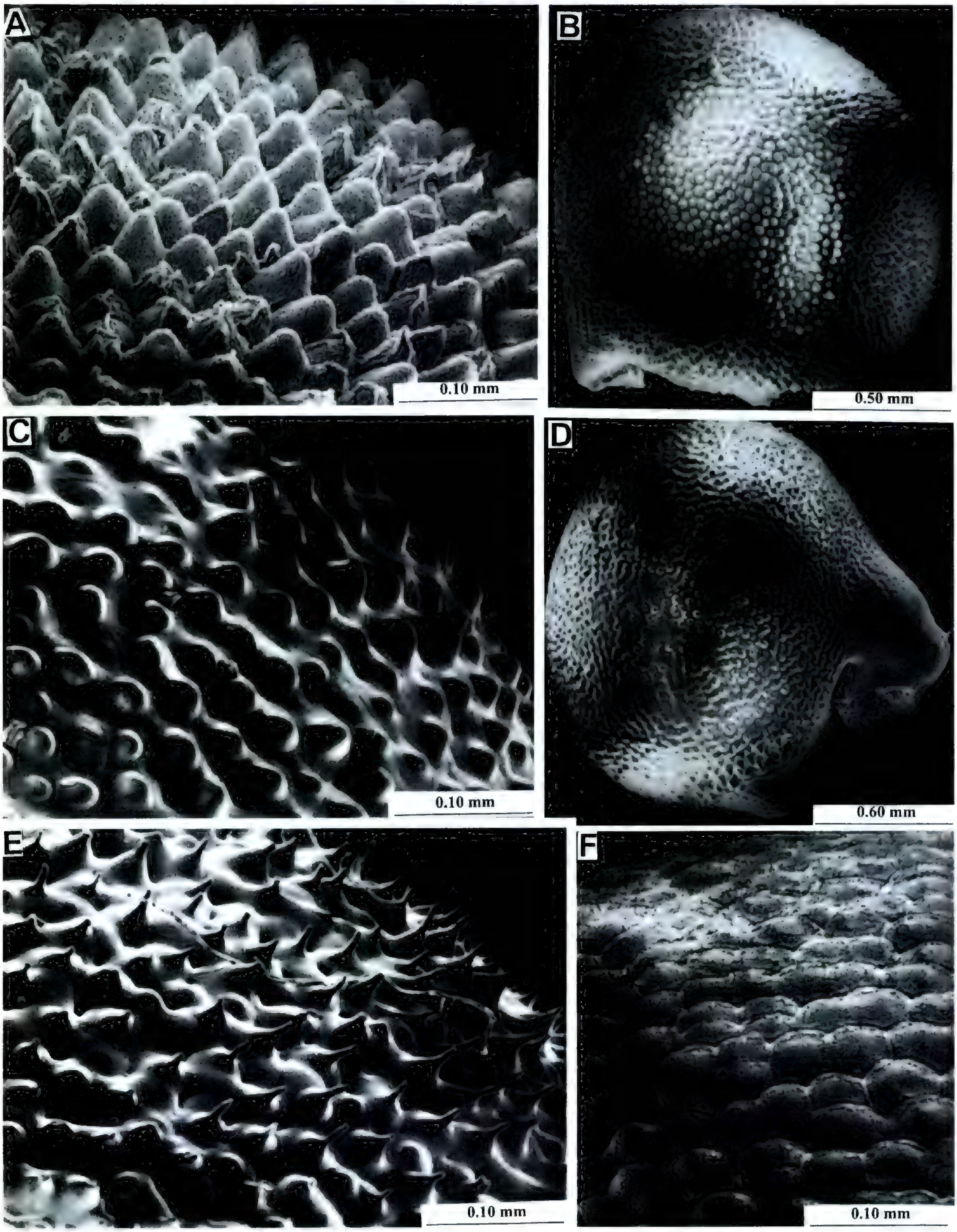


Figure 10. A, B. *Hypoxis subspicata.* —A. Seed coat sculpture. —B. Seed. C & D. *Hypoxis hockii* var. *hockii.* —C. Seed coat sculpture. —D. Seed. —E. *Hypoxis hockii* var. *katangensis,* seed coat sculpture. —F. *Hypoxis hockii* var. *colliculata,* seed coat sculpture. A and B from *Duvigneaud & Timperman 2528* (BRLU); C and D from *Hock s.n.* (BR); E from *Lisowski, Malaisse & Symoens 11797* (POZG); F from *Lisowski, Malaisse & Symoens 7682* (POZG).

flat verrucose papillae (Fig. 10F) are a stable character distinguishing this taxon.

8c. Hypoxis hockii var. **katangensis** (Nel ex De Wild.) Wiland, comb. et stat. nov. Basionym: *Hypoxis katangensis* Nel ex De Wild., Bot. Jahrb. Syst. 51: 312. 1914. TYPE: Congo-Kinshasa. "Oberer Kongo-Bezirk: Katanga," 1900, *Verdick s.n.* (holotype, BR!). Figures 9G–J, 10E.

Herb to 36 cm high. *Outer leaves* wide elliptical 3.6–7.8 × 4–5 cm, pubescent on whole surface below with long white two-branched or tufted trichomes, sparsely pubescent in apical part above; nerves 27 to 39; *inner leaves* 5 to 15, linear, 10.5–35.0 × 0.8–14 cm, prominently pubescent on margins and the midrib below with tufted trichomes, on surface only sparsely pubescent with 2- or 3-branched trichomes, in basal part only ciliate; nerves 25 to 39. *Scapes* 5 to 12, 7–34 cm × ca. 3 mm wide, pubescent with golden tufted trichomes; raceme 10- to 13-flowered; *bracts* 13–20 × 1–2 mm wide, the lowermost usually 1-nerved, rarely 3-nerved; *pedicels* 7–15 mm long. *Tepals* 6; *outer tepals* 10–12 × 4–5 mm wide, 5- to 9-nerved; *inner tepals* broadly ovate, obtuse at apex, 10–11 × 4–7 mm wide, 7-nerved, pilose abaxially on lower two thirds of midrib; *stamens* 7–10 mm long; *filaments* almost linear, ca. 3.5 mm long; *anthers* 4.5–8.0 mm long, slightly or not emarginate at apex; *ovary* 3.5–4.0 × 3.5–4.0 mm wide; *style* ca. 4 mm long; *stigma* ca. 2 mm long, composed of 3 fused lobes and conical or composed of 3 free lobes, obtuse at apex. *Capsule* 6–7 × 4–5 mm; *seeds* ovoid, ca. 1.5 × 1 mm wide, black and glossy; seed coat with long-acuminate conical papillae.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Plateau des Marungu, Mont Kibobwa, près du village Kibobwa, *Lisowski, Malaisse & Symoens 11797* (POZG). ZAMBIA. Kalungwisi Flüsschen, *Fries 1148* (Z).

Hypoxis hockii var. *katangensis* is known only from a few collections. Seeds with long-acuminate conical papillae (Fig. 10E) and less dense indumentum of the leaves distinguish this taxon from other varieties.

9. Hypoxis kilimanjarica Baker, in Dyer, Fl. Trop. Afr. 7: 378. 1898. TYPE: Tanzania. "Marangu, Lager am Ruassibach, Bergwiese oberhalb des Urwaldes, 2440 m, 31.08.1893," *Volkens 781* (holotype, B!; isotypes, K!, BM!). Figures 11A, 12A, B.

Hypoxis incisa Nel, Bot. Jahrb. Syst. 51: 301. 1914. TYPE: Tanzania. Lutindi, 1900, *Liebusch s.n.* (lectotype, designated by Nordal et al. (1985), B!).

Herb to 20 cm high; *rhizome* subglobose, 6–14 × 6–13 mm diam. (dried out); tunic black membranous, to 2.5 cm high, occasionally with some soft fibers; roots not very thick. *Outer leaves* ca. 2, grouped in short pseudostem, oblong, acute, ca. 3 cm × 4 mm wide, ciliate on margins or glabrous; nerves unequal, ca. 7; *inner leaves* 3 to 8, narrow-linear, reflexed to prostrate, often spathe-like in basal part, 3.5–22.0 cm × (1–) 2–4 mm, sparsely pilose on margins and midrib below, sometimes also on the blade surface; trichomes 2- to 3-branched; nerves unequal, 9 to 13, some of them very thin. *Scapes* 1 to 4, 4–16 cm × 0.3–1.0 mm, usually bending downward after flowering, narrowly winged and ciliate at base, in upper half wider and pubescent; trichomes 2-branched, golden or red-brown; *flowers* single or 2, often the second bud remaining undeveloped; *bracts* single, rarely 2, subulate, very narrow, acute, 3–10 × 0.4–1.0 mm, pubescent abaxially with 2-branched trichomes along nerve; *pedicels* 2.5–6.0 mm long, pubescent with 2- to 5-branched trichomes. *Tepals* 6, exceptionally 3 or 4; *outer tepals* elliptic, acute, 5–7 × 2–3 mm, pubescent abaxially with 2- to 5-branched trichomes, irregularly 7-nerved; *inner tepals* elliptic or ovate, cuspidate, 4–7 × 1.5–3.0 mm, pubescent along lower ¾ of midrib abaxially, irregularly 5-nerved; *stamens* unequal; outer stamens 3–4 mm long with filaments 2–3 mm long; inner stamens 2.5–3.5 mm long with filaments 1.5–2.0 mm long; *filaments* subulate; *anthers* 1.5–2.0 mm long, sagittate, fused and obtuse at apex; *ovary* obconical, 2–4 × ca. 1.5–2.0 mm, pubescent; *style* wider in basal part, trigonous, 1–2 mm long; *stigma* pyramidal, obtuse at apex, with three stripes of papillae, 1–2 mm long; proportion of length of style to length of stigma various. *Capsule* obovoid, 4–8 × 2.5–3.5 mm, almost glabrous; *seeds* numerous, ovoid, ca. 1.5 × 1 mm, black; seed coat colliculate.

Distribution and ecology. Congo-Kinshasa, Rwanda, Burundi (Fig. 22), Kenya, Tanzania. For Central Africa this species was previously reported from Congo-Kinshasa (as *H. incisa* Nel in Robyns & Tournay, 1955) and from Rwanda (Champluvier, 1987), though it also occurs in Burundi. Montane woodland, montane grassland, often associated with *Erica*; on rocky, moist soils; alt. 1850–2950 m. Flowering from January to March, from May to June, and from August to October.

Additional specimens examined. CONGO-KINSHASA. **Haut-Zaïre:** Awo, Mt. Aboro, *Froment 681* (BR). **Kivu:** Mt. Kahuzi, *Bamps 2878* (BR, K); Buzezu, *Hen-*

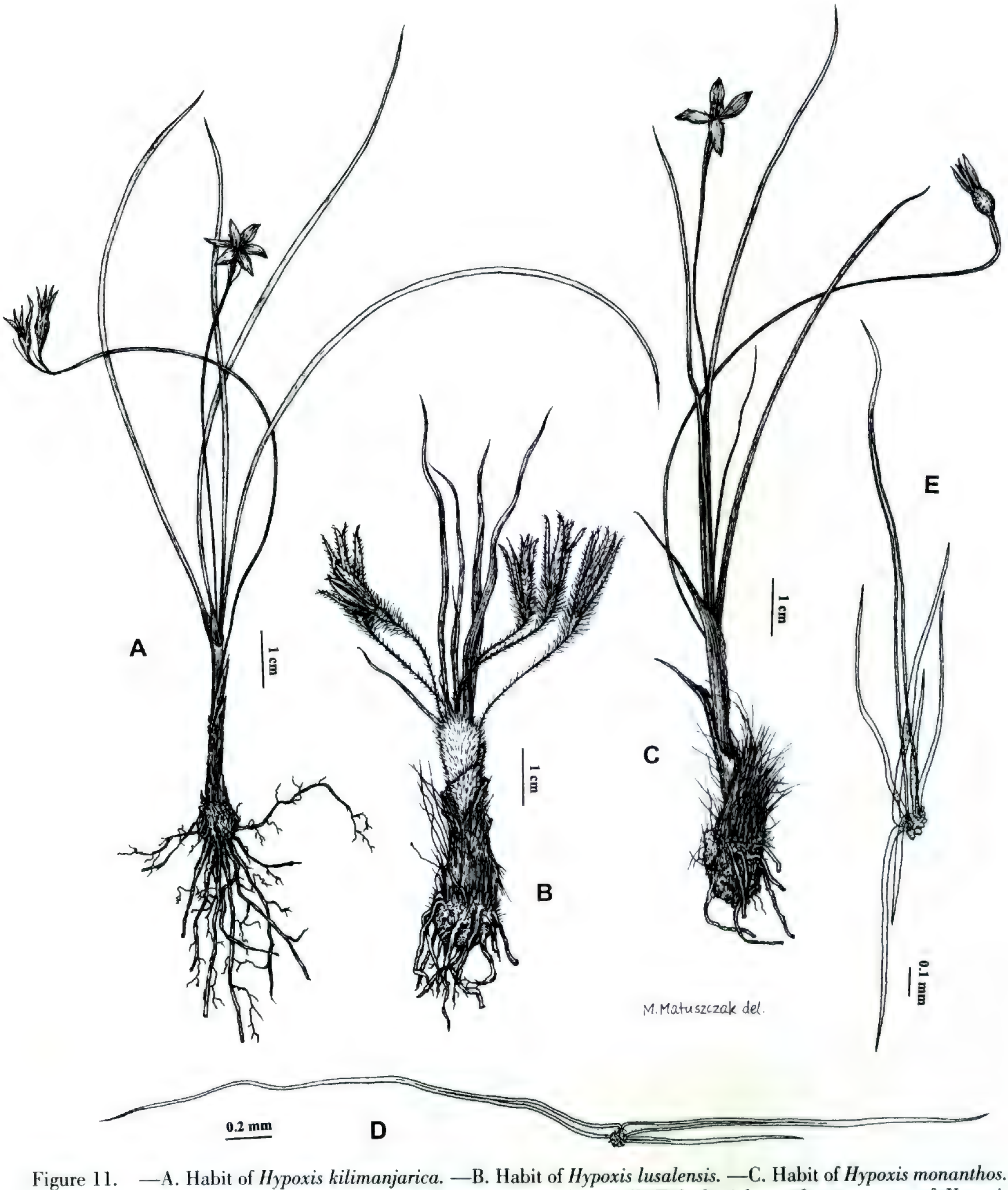


Figure 11. —A. Habit of *Hypoxis kilimanjarica*. —B. Habit of *Hypoxis lusalensis*. —C. Habit of *Hypoxis monanthos*. —D. Three-branched trichome from a scape of *Hypoxis monanthos*. —E. Tufted trichome from a scape of *Hypoxis lusalensis*. A from *Lewalle 3997* (BR); B from *Lisowski, Malaisse & Symoens 8453* (POZG); C from *Lisowski B7173* (POZG); D from *Lisowski, Malaisse & Symoens 10710* (POZG); E from *Lisowski, Malaisse & Symoens 8557* (POZG).

drickx 4780 & 4832 (BR). RWANDA. Cyangugu, Mt. Bigugu, *Bamps 3302* (BR), *Christiaensen 1620* (BR); forest of Nyungwe, *Bouxin 429 & 984* (BR); Rugege, *Christiaensen 1501* (BR). BURUNDI. Muramwya, Mt. Manga, *Reekmans 756* (BR) & *2065* (BR, MO); *Lewalle 584 & 1102* (BR); Teza, *Reekmans 1335* (BR, MO), *Smets 13* (BRLU); Nyabigondo-Teza, *Lewalle 3997* (BR); Teza, Mt. Ngoma, *Lejoly 84/719* (BRLU); Bururi, Tora, *Reekmans 23* (BR, MO), *Lewalle 5130* (BR, MO); Luvironza, INEAC, *Michel 5007* (BR).

Vernacular name. Bukanga-Tshiragaga (dialect Bashi).

Hypoxis kilimanjarica is primarily an East African species. Studied by Nordal et al. (1985) it is divided into *H. kilimanjarica* subsp. *kilimanjarica* and *H. kilimanjarica* subsp. *prostrata* Holt & Staubo. In Central Africa only the typical subspecies with erect or slightly reflexed leaves and scapes

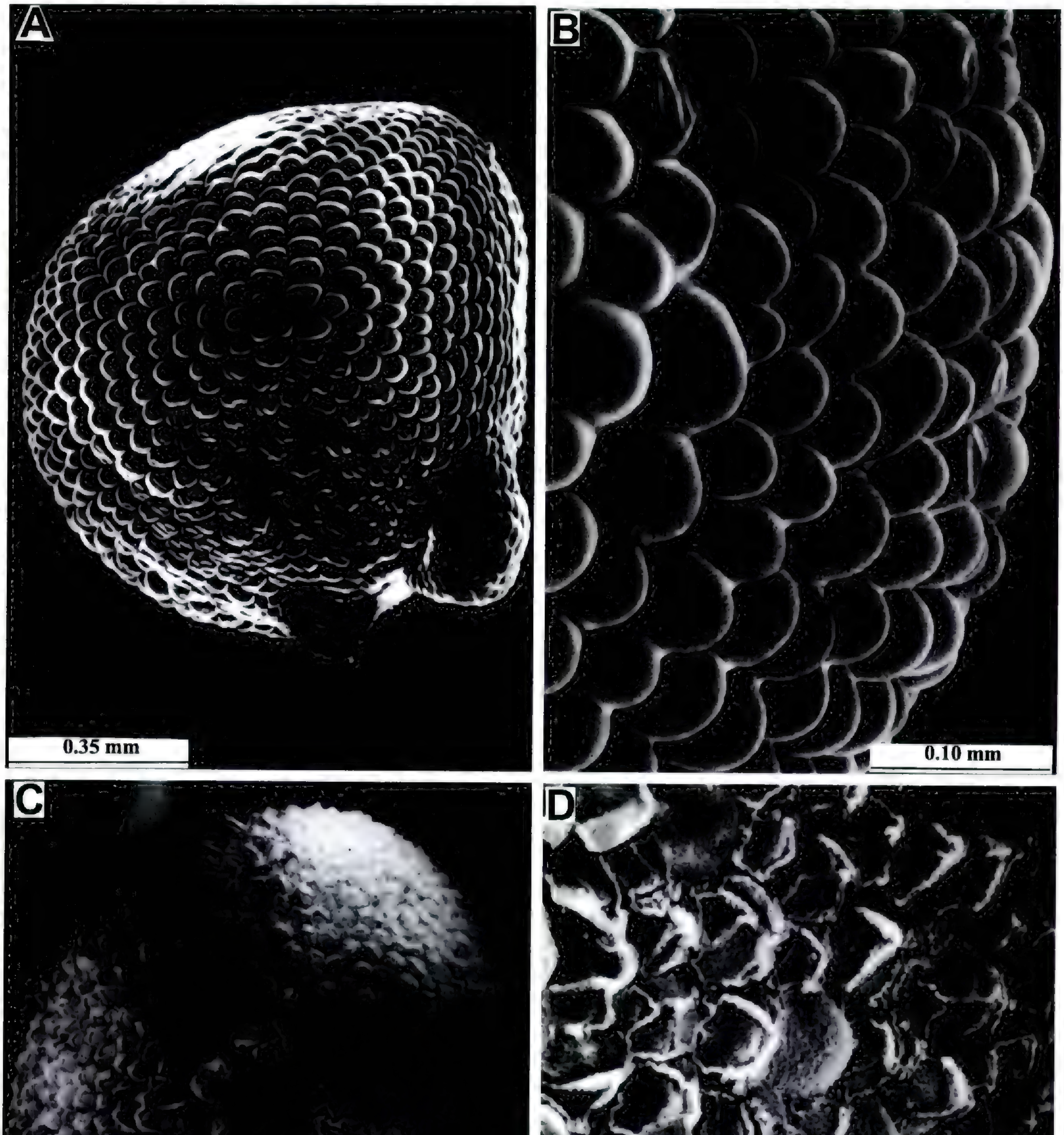


Figure 12. A, B. *Hypoxis kilimanjarica.* —A. Seed. —B. Seed coat sculpture. C, D. *Hypoxis monanthos.* —C. Seed. —D. Seed coat sculpture. A and B from *Bouxin 984* (BR); C and D from *Lisowski B 7173* (POZG).

(Fig. 11A) more than 4 cm long occurs. The other subspecies bears prostrate leaves and scapes never exceeding 4 cm.

Leaves of *H. kilimanjarica* were sometimes referred to as canaliculate (Nel, 1914b), but according to my observations, they are rather carinate. This feature as well as 9 to 13 veins on inner leaves usually about 2 mm wide distinguish this species from the slightly similar *H. filiformis* (Fig. 6D), which possesses canaliculate leaves, 0.7 mm wide and with 3 nerves. *Hypoxis kilimanjarica* occasionally resembles *H. monanthos*, especially those plants with only 4 tepals. The scapes in both species bend downward after flowering (Fig. 11A, E). *Hypoxis kilimanjarica* is, however, a montane species and occurs above 1850 m, whereas *H. monanthos* only rarely reaches this altitude and is found in very moist habitats. Seeds of the former are black and covered with thin cuticle (Fig. 12A, B), and of the latter brown and coated with thick cuticle (Fig. 12C, D). From *H. angustifolia, H. kilimanjarica* is distinguished by shorter pedicels, only to 6 mm long, bending scapes, and black seeds. *Hypoxis angustifolia* bears pedicels at least 12 mm long, erect scapes, and brown seeds (Fig. 3).

10. Hypoxis lejolyana Wiland, Fragm. Florist. Geobot. 42: 418. 1997. TYPE: Congo-Kinshasa. Shaba: "route Dilolo-Kisenge, Kahundu, source de la Lovoa, Dilolo poste-Dilolo station," Sep. 1958, *Plancke 158/2184* (holotype, BRLU!). Figure 13A, B.

Herb to 29 cm high; *rhizome* ovoid, ca. 4 cm diam.; tunic stiff, fibrous, with 2–3 mm wide blades often covered with indumentum, to 7 cm; roots partially terete. *Outer leaves* 2 to 3, ca. 5 cm × 8 mm, basally with membranous margins, linear, pubescent on upper half with 2-branched white or yellow trichomes; nerves unequal, ca. 13; *inner leaves* 3 to 5, linear, prominently keeled, to 29 cm × ca. 3 mm, ciliate on margins and midrib with tufted trichomes, hispid beneath and sparsely pilose above with 2-branched trichomes; nerves unequal, ca. 9. *Scapes* 2 to 4, to 25 cm × ca. 2 mm, winged and glabrous in lower half, wider and pubescent in upper half; *cyme* 2- to 5-flowered; in 5-flowered inflorescences, 3 flowers are clustered at the top, with one sessile and two pedicellate; floral anthesis basipetal; *bracts* subulate, 5–15 × 1 mm, single-nerved, pubescent on midrib abaxially; *pedicels* 2–15 mm long. *Tepals* 6; *outer tepals* ovate, 7.5–8.0 × ca. 3 mm, 7-nerved, pubescent abaxially; *inner tepals* ovate, acute, 7 × ca. 2.5 mm, 5-nerved, pilose along midrib abaxially; *stamens* unequal; outer stamens ca. 3.5 mm long with filaments ca. 1.5 mm long; inner stamens ca. 2.5 mm long with filaments ca. 1 mm long; *filaments* subulate; *anthers* linear, deeply sagittate, retuse at top, ca. 2 mm long; *ovary* obconical, ca. 4 × 3–4 mm wide, pubescent; *style* ca. 2 mm long; *stigma* conical, ca. 1 mm long, composed of 3 lobes with margins covered with numerous papillae. *Capsule* turbinate, 6–7 × ca. 4 mm, pubescent; *seeds* ovoid, ca. 2 × 1.5 mm, brown; cuticle thick, brown-black; seed coat with pyramidal pointed projections.

Distribution and ecology. A taxon known from Shaba Province in Congo-Kinshasa (Fig. 27) and from one collection in Zambia. Wooded grassland with *Parinari* sp.; pasturage with natural vegetation; on Kalahari sand. Flowering from August to October and in December.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Tschibalaka, *Overlaet 712* (BR); Mangoa, 20 km E de Dilolo-Gare, *Duvigneaud & Timperman 2519* (BRLU); Plateau de la Manika, *Lisowski, Malaisse & Symoens 178* (POZG), *Duvigneaud 4532 H* (BRLU). ZAMBIA. **Western Province:** Kalabo Dist., 2 mi. W of Kalabo, *Drummond & Cookson 6431* (MO).

The tunic covered with indumentum and an inflorescence with basipetal flower anthesis distinguish *H. lejolyana* from all Central African species with leaves not exceeding 8 mm and scapes 1–2 mm wide. Especially the unique shape of the 5-flowered inflorescence with 3 flowers clustered at the top, 2 being pedicellate and 1 sessile, is worth noticing. From *H. subspicata*, a species with a similar but much larger inflorescence, *H. lejolyana* is distinguished by its smaller flowers with tepals to 8 mm long, and inner leaves 3 mm wide. Tepals of *H. subspicata* are longer, to 13–15 mm, and its inner leaves are narrower, to 0.8–2.0 mm wide.

11. Hypoxis lusalensis Wiland, Fragm. Florist. Geobot. 42: 421. 1997. TYPE: Congo-Kinshasa. Shaba: "Plateau des Marungu, 20 km au NNE de Kasiki, sommet Lusale," 2450 m, Nov. 1969, *Lisowski, Malaisse & Symoens 8453* (holotype, POZG!). Figures 7C, D, 11B, E.

Herb to 24 cm high; *rhizome* globose, 1.5 cm diam. (dried out); tunic membranous and fibrous, 2–5 cm long. *Outer leaves* 3 to 5, subulate, 2.5–8.5 × ca. 1.4 cm, glabrous at base, apically pubescent below; indumentum gray; trichomes tufted, ca. 6-branched, 0.4–3.0 mm long; nerves unequal 9 to 13; *inner leaves* 4 to 6, narrow-linear, 5–24 cm × 2–3 mm, villous-hispid on margins and midrib below, sparsely pilose above; nerves unequal, 7 to 9. *Scapes* 2 to 4, 5–12 cm × 1.0–1.5 mm, sparsely

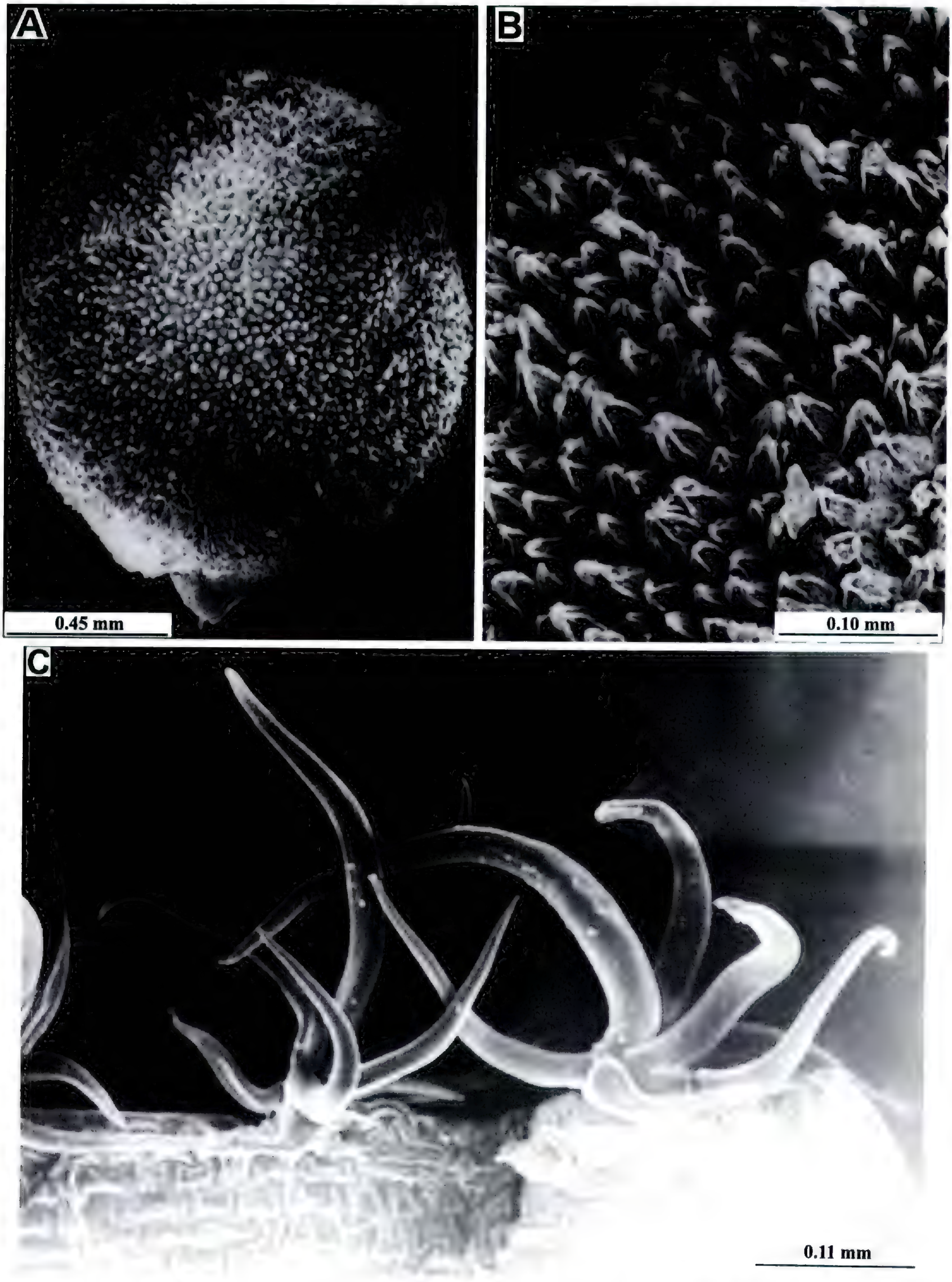


Figure 13. A, B. *Hypoxis lejolyana.* —A. Seed. —B. Seed coat sculpture. —C. *Hypoxis goetzei,* tuft trichomes on a leaf margin. A and B from *Plancke 158/2184* (BR); C from *Quarré 704* (BR).

ciliate in basal half, prominently hispid in upper half; trichomes tufted; *flowers* 1 or 2; *bracts* lanceolate or ovate, acute at apex, 2–12 × 1–3 mm, hispid abaxially; *pedicels* 6–14 mm long, elongating with age, hispid. *Tepals* 6; *outer tepals* lanceolate or elliptic, 14–20 × 3.5–4.0 mm, hispid abaxially, 5- to 7-nerved; *inner tepals* elliptic, cuspidate, 13–18 × 4–5 mm, abaxially hispid on midrib in lowest third (or ⅔), 5-nerved; *stamens* equal, 7 mm long; *filaments* subulate, 3.5–4.0 mm long; *anthers* narrow-linear, sagittate, fused at apex, ca. 4 mm long; *ovary* obovoid, 5.0–5.5 × 2.5–3.0 mm, long hispid; *style* triangular, 1.5–2.0 mm long; *stigma* composed of 3 oblong lobes, obtuse at apex, 2.5–4.0 mm long. *Capsule* fusiform, ca. 8 × 3.5 mm, villous; *seeds* numerous, irregularly ovoid, ca. 1 × 1 mm, black-brown; cuticle thick; seed coat with pointed projections.

Distribution and ecology. Species known only from Plateau des Marungu in Congo-Kinshasa (Fig. 22), where it occurs in grasslands and river valleys; alt. 1700, 2320–2450 m. Flowering November.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Plateau des Marungu, pente N de la montagne Lusale, *Lisowski, Malaisse & Symoens 8557* (POZG); Mt. Lusale, *Lisowski, Malaisse & Symoens 8300 & 8684* (POZG); Marungu, section Mulongoshi, *Quarré 7252* (BR) pro parte quoad "c."

Hypoxis lusalensis is very easy to recognize among Central African taxa due to its flowers with long tepals to 20 mm, large relative to its small stature (Fig. 11B), and 2–3 mm wide inner leaves covered with long, gray hispid indumentum. Given its morphological uniqueness, further studies will be needed to determine its relationships to other species.

12. Hypoxis malaissei Wiland, Fragm. Florist. Geobot. 42: 418. 1997. TYPE: Congo-Kinshasa. Shaba: "28 km au N.E. de Lubumbashi," alt. 1208 m, Apr. 1971, *Malaisse 7403* (holotype, BR!). Figure 14A, B.

Herb to 16.5 cm high; *rhizome* globose, ca. 10 mm diam. (dried out); tunic membranous and fibrous, black, to 2 cm. *Outer leaves* ca. 2, ovate, ca. 10 mm wide, spathe-like in basal part, pubescent beneath with tufted trichomes; *inner leaves* ca. 4, linear, 7.5–16.5 cm × 2.5–4.0 mm, hispid; trichomes 2-branched, white; nerves unequal, 2 as large as midrib, ca. 7. *Scapes* 1 to 3, ca. 9.5 cm × 1.5 mm, hispid, especially in upper half, with long white tufted trichomes; *flowers* 2, sometimes 1 flower developed and 1 remaining as an undeveloped bud; *bracts* subulate, ca. 5.5 × 1 mm, hispid along midrib abaxially; *pedicels* 7–12 mm long, hispid. *Tepals* 6; *outer tepals* elliptic, keeled, 7.5 × 2.5 mm, densely hispid abaxially, irregularly 5-nerved; *inner tepals* obovate, acute at apex, 6.5 × 2.5 mm wide, hispid abaxially on midrib on lowest ⅓, irregularly 5-nerved; *stamens* equal, ca. 4 mm long; *filaments* filiform, ca. 2.5 mm long; *anthers* ca. 2 mm long, sagittate, fused and obtuse at apex; *ovary* obconical, ca. 3.5 × 2.5 mm wide, hispid; *style* slender, ca. 1.5 mm long; *stigma* ca. 1 mm long, composed of 3 very thin rows of papillae, obtuse at apex. *Capsule* obovoid, ca. 5 × 3.5 mm, hispid; *seeds* several, ovoid, 2 × 1 mm wide, black; seed coat honeycombed.

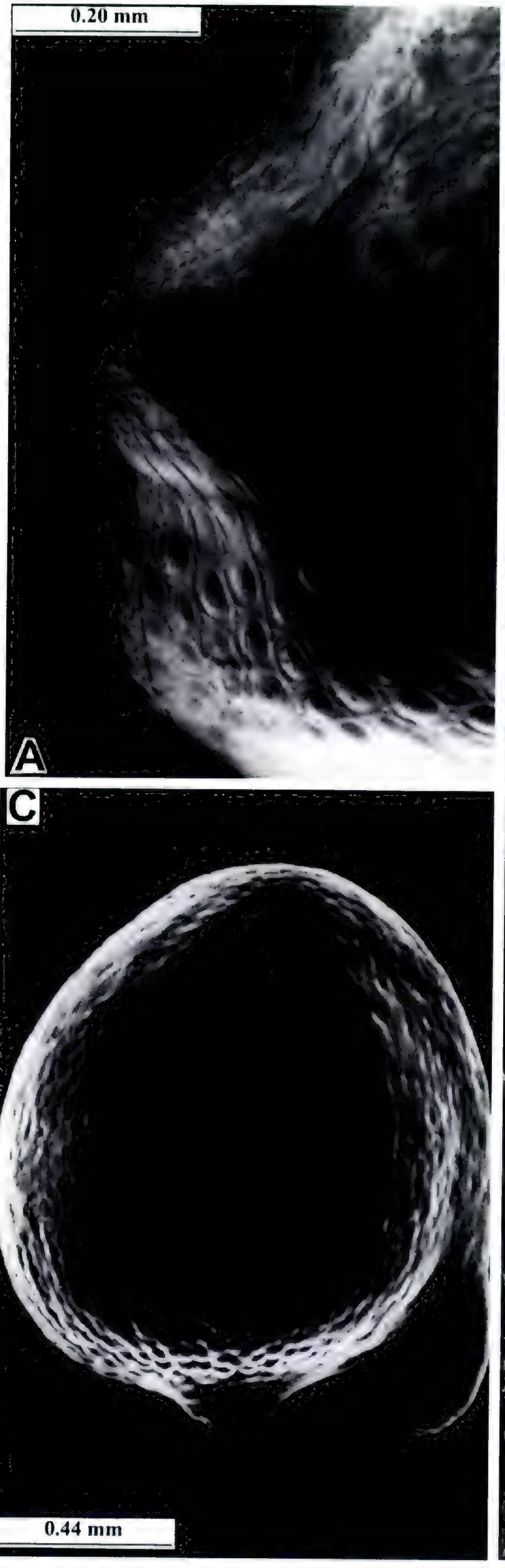
Distribution and ecology. Endemic to Shaba Province of Congo-Kinshasa (Fig. 22), *H. malaissei* is known only from a single collection in miombo; alt. 1208 m. Flowering in April.

Hypoxis malaissei closely resembles *H. muhilensis*, which occurs further north on Plateau des Muhila. Both species share a robust habit and partially fibrous tunic, which distinguishes them from slender *H. angustifolia* with its membranous tunic. However, *H. malaissei* possesses black seeds with a thin cuticle displaying a most unique honeycombed seed coat sculpture (Fig. 14 A, B). The two aforementioned species bear brown seeds, covered with thick cuticle (Figs. 3, 17). More collections will be needed to determine the taxonomic position and relationships of *H. malaissei* to other species.

13. Hypoxis malosana Baker, Bull. Misc. Inform. 1897: 284. TYPE: Malawi. "Mount Malosa, near Zomba," alt. 4000–6000 ft., *Whyte s.n.* (holotype, K!). Figures 15A–C, 16.

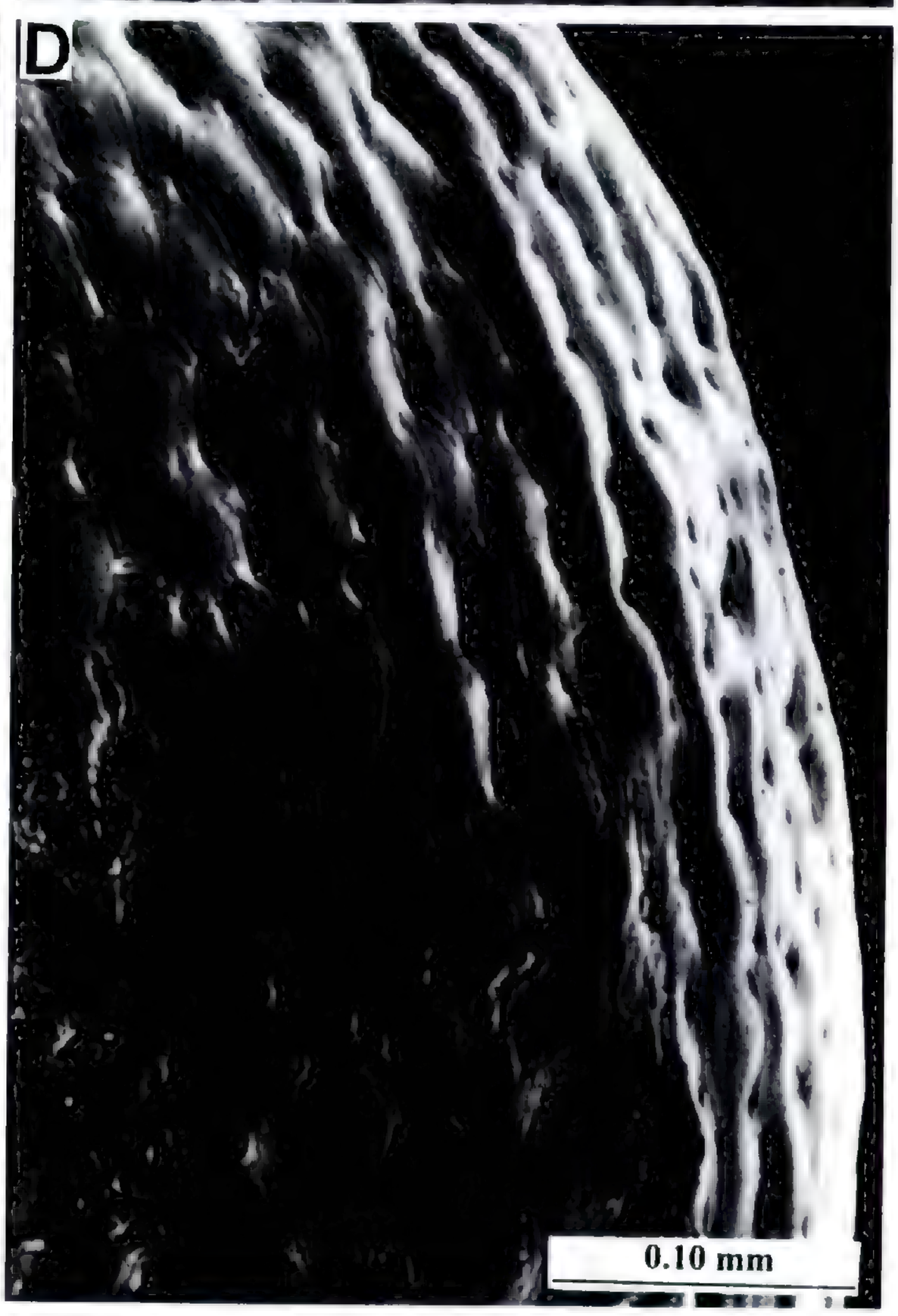
Hypoxis biflora De Wildeman, Repert. Spec. Nov. Regni Veg. II: 537. 1913. TYPE: Congo-Kinshasa. "Ober-Katanga," Oct. 1911, *Hock s.n.* (holotype, BR!).

Herb to 20–50 cm high; *rhizome* ovoid, 1.0–4.5 × 1.1–4.0 cm (dried out); tunic fibrous, stiff black, to 6 cm high. *Outer leaves* 1 to 3, ovate, 3.0–14.5 cm × 4–10 mm, pilose below; trichomes 2-branched; nerves unequal, 7 to 13; *inner leaves* 5 to 11, narrow-linear, 2.8–61.0 cm × 1–3 mm wide, hispid on margins and midrib below; trichomes 2-branched, ca. 3 mm long, falling off with age, red-brown or golden; nerves unequal 5 to 11. *Scapes* 2 to 10, 2.5–57.0 cm × 0.5–1.0 mm, often red tinted, in basal half ciliate with 2-branched trichomes, in upper half pubescent with tufted trichomes; trichomes white to red-brown, 0.4–1.3 mm long; *cyme* corymbiform, 2- to 4-flowered, with pedicels of significantly various length; *bracts* subulate, 2–8 × ca. 1 mm, pubescent along midrib abaxially; *pedicels*


0.20 mm
A

0.10 mm
B

C
0.44 mm


D
0.10 mm

from almost none at lowest flowers in the inflorescence to 23 mm long at upper, pubescent. *Tepals* (5) 6; *outer tepals* elliptic, 6–12 × 2–3 mm; appendage minute, pubescent abaxially, with 5 to 7 parallel nerves; *inner tepals* oblong or ovate, acute at apex, 5.0–11.5 × 2–4 mm, almost glabrous with few trichomes at base abaxially, with 3 to 7 parallel nerves; *stamens* unequal; outer stamens 2.5–5.0 mm long with filaments 2.0–3.5 mm long; inner stamens 1.5–3.0 mm long with filaments 0.5–1.5 mm long; *filaments* filiform; *anthers* 1.5–2.5 mm, sagittate, emarginate at apex; *ovary* obconical, 2.0–3.5 × 1.5–2.5 mm, pubescent; *style* to 0.5 mm long, or stigma sessile; *stigma* oblong or pyramidal, obtuse, composed of 3 oblong lobes, entirely fused or emarginate at apex, 1.3–2.2 mm long. *Capsule* obovoid, 3–5 × 2–3 mm, pubescent, opening by transverse slit; *seeds* numerous, ovoid, ca. 1.5 × 1 mm, black; seed coat prominently colliculate.

Distribution and ecology. Widely distributed in Congo-Kinshasa and Burundi (Fig. 23), Tanzania, Zambia, Malawi, Zimbabwe, Mozambique, Republic of South Africa. Occurs in miombo, wooded grassland, moist or temporarily inundated grasslands, swamps, dembo, wet river valleys, pastures. Soils moist black, boggy, loamy; gray, kaolin soils; sandy soil; white soils; granite bed; soils with copper; alt. 1000–1810 (–2450) m. Flowering mainly from August to February, sometimes from April to June.

Additional specimens examined. CONGO-KINSHASA. **Kivu:** Luemba, *Kinet 49 (=Hendrickx 4361)* (BR). **Shaba:** s. l., *Hock s.n.* (BR); 10°46′30″S, 25°17′E, *Schaijes 3032* (BR); Kambove, Kisungu, *Streel 331* (BR); Dikuluwe, *Duvigneaud 4129 & 4130H,* (BRLU); Lubumbashi, route de Sakania, *Duvigneaud 4312 & 4861* (BRLU); Kisanga [= Keyberg], 7 km SSO d'Elisabethville, *Schmitz 2109* (BR); vallée Kimilolo, *Detilleux 383* (BR); Kapeluka, 20 km NO d'Elisabethville, *Schmitz 7675* (BR); 28 km NO d'Elisabethville, *Schmitz 5855* (BR); Dilungu à 9 km de Dilolopost vers Katundu, rivière Mokwegi près de Kandala, *Plancke 159/2206 & Plancke 159/2214* (BRLU); environs de Lubumbashi, bord de la Natwebo, *Lisowski 81124 & 81146 & B-7173B & B-7270A & B-7271 & B-7279A* (POZG); au SE de Lubumbashi, près de la Mission Don Bosco, *Lisowski 82460 & B-7272* (POZG); environ 30 km au N de Lubumbashi, près de la ferme St. Hubert, *Lisowski 81145* (POZG); Kipopo, 25 km ONO d'Elisabethville, *Schmitz 4198* (BR), *Symoens 13672* (BR); Kaponda, *Malaisse 13562* (BR, WAG); à 1.5 km à l'E de Kabiashia, *Malaisse 6100* (BR); Katuba, *Quarré 719 & 3717 & 4314* (BR); vallée de la Karavia, *Quarré 3527* (BR); environs de Kabiashia, près du village Kimangu, *Lisowski 81109 & Lisowski 81121* (POZG); vallée de Luapula, près de Kiniama, *Lisowski 81122 & Lisowski B-7274 & Lisowski B-7277* (POZG); Gombela, *Lisowski B-7231* (POZG); environs de Tshinsenda, *Lisowski B-7273* (POZG); domaine de Muhila, au-dessus de Kalobele, *Lisowski 7274* (POZG); vallée de la Potopo, *Schaijes 2398* (BR); P.N. Upemba, *Heine 108 & U 111* (BR), *de Witte 7181* (BR); Mukana, *de Witte 2741* (BR); Kibara, entre Buyabalo et Katonga, *Robyns 3604* (BR); Mitwaba, *Duvigneaud & Timperman 2697* (BRLU); Plateau des Kundelungu, *Lisowski, Malaisse & Symoens 1155 & 7470 & 7596* (POZG); à 3 km au S de la source occidentale de la Lutshipuka, *Lisowski, Malaisse & Symoens 7744* (POZG); près de la source de la Lutshipuka, *Lisowski, Malaisse & Symoens 810* (POZG); 3 km à W du Poste Lualala, *Lisowski, Malaisse & Symoens 7477* (POZG); contreforts des Kundelungu, vallée de la Kapolo, près du village Kibuzi, *Lisowski 92473* (POZG); Plateau du Biano, *Duvigneaud 1319* (BRLU); au S de Biano-Gare, *Duvigneaud 1324* (BRLU); Kiankwali, *Duvigneaud 4471* (BRLU); Dilungu-Yulu entre Nzilo et Kensenia, *Duvigneaud & Timperman 2626* (BRLU); près de la gare Kansenia, *Lisowski, Malaisse & Symoens 13531* (POZG); au N du Tenke, *Duvigneaud 1311* (BRLU); Plateau des Marungu, section de Mulongoshi, *Quarré 7256* (BR); à 3 km au SE du Poste Mulungoshi, *Lisowski, Malaisse & Symoens 8125 & 8134A* (POZG); sommet Lusale, à 10 km au NNE de Kasiki, *Lisowski, Malaisse & Symoens 8369* (POZG); environs de Kasiki, mare Kasozia, *Lisowski, Malaisse & Symoens 12058A* (POZG); à env. 18 km de Pepa, *Symoens 779* (BRLU); Elgymapepa, *Kisimba, Muzinga & Matamba 136* (BR); à 5 km à l'W de Niembe, *Lisowski 8023 & 82893* (POZG); Plateau de la Manika, Katentania, *Homblé 809* (BR), *Doumen 9* (BR); entre Kolwezi et Djoni, *Lisowski, Malaisse & Symoens 7822* (BR, POZG); Plateau de la Mangoa, Dilolo, *Duvigneaud & Timperman 2404 & 2406* (BRLU). BURUNDI. Nyakazu, *Rameloo 4775* (BR); Bururi, *Becquet 2153* (BR); Rutana, *Staner 2077* (BR).

Vernacular name. Ngandama (Luemba, *Kinet 49*).

Hypoxis malosana is a common and easy- to-distinguish species with a characteristic large, black, stiff, and fibrous tunic (Fig. 15A, B). Its other characteristic is the inflorescence with pedicels greatly varying in length. Its rhizome is edible.

In Central Africa *Hypoxis filiformis* might be confused with *H. malosana,* seen as an undeveloped exemplar of the latter (Fig. 6D). *Hypoxis malosana,* however, always bears a prominent tunic, while the tunic of *H. filiformis* is mainly membranous, with only few soft fibers. It seems, however, that in South Africa such species as *H. malosana, H. junodii* Baker, and *H. caespitosa* Baker have overlapping morphological variability, being very similar in ap-

←

Figure 14. A, B. *Hypoxis malaissei.* —A. Micropylar end of the seed. —B. Seed coat sculpture. C & D. *Hypoxis robusta.* —C. Seed. —D. Seed coat sculpture. A and B from *Malaisse 7403* (BR); C and D from *Duvigneaud & Timperman 2213* (BRLU).

M Matuszczak del.

pearance with black colliculate seeds (Fig. 16) and prominent tunics. Therefore, this complex of species is in need of thorough evaluation in this region to establish relationships among them, as well as between this group and *H. filiformis*. *Hypoxis filiformis* and *H. malosana* were confused in the past (De Wildeman, 1921; Zimudzi, 1996), and it is certain that more investigation of their relationship is needed.

For the flora of Central Africa, *H. malosana* was described by De Wildeman (1913) as *Hypoxis biflora*. This name was illegitimate when published, being a later homonym of *H. biflora* Baker (Baker, 1876). Later Baker (1878a) reduced his *H. biflora* to taxonomic synonymy under *H. angustifolia* Lam. Nel (1914b) invalidly recombined De Wildeman's illegitimate species name as "*H. dregei* Baker" var. *biflora* (De Wild.) Nel. Nel mistakenly listed *H. dregei* Baker at the rank of species, whereas Baker (1878a) had only used the epithet "*dregei*" at the rank of variety, as *H. sericea* Baker var. *dregei* Baker. *Hypoxis sericea* (syntype, *Drege 8525*, K!) possesses longer, silky adpressed indumentum on the leaves, very different from the hispid indumentum of *H. malosana*. This species does not occur in Central Africa. However, the type specimen of *H. biflora* is a specimen of *H. malosana*. Similar observations were stated by Nordal et al. (1985).

14. Hypoxis monanthos Baker, Trans. Linn. Soc. ser. 2: 266. 1878. TYPE: Angola. Huilla, *Welwitsch 4058* (holotype, BM!). Figures 11C, D, 12C, D.

Herb to 16 cm high; *rhizome* subglobose 13–17 × 10–13 mm (dried out); tunic fibrous and membranous, to 3.5 cm high, sometimes absent. *Outer leaves* 3–4, ovate, tapering toward apex, 2–20 cm × 6–10 mm, ciliate on margins; trichomes 2- to 3-branched; nerves unequal, 7 to 9; *inner leaves* 3 to 9, narrow-linear, keeled, often reflexed, 11–34 cm × 2–4 mm, sparsely pilose on margins and midrib below, sometimes also on the blade surface abaxially, with 2- to 3-branched trichomes falling off with age; nerves unequal 5 (7), some of them very thin. *Scapes* 1 to 4, 7–20 cm × 0.5–1.0 mm, narrowly winged and ciliate at base, villous above, usually bent downward after flowering; trichomes 2-branched or tufted, gray or golden; *flowers* single or 2; *bracts* single, rarely 2, subulate, 3–10 × 0.4–1.0 mm, pubescent along midrib abaxially; *pedicels* 0–5 mm long, villous. *Tepals* 4 or 6; *outer tepals* elliptic, 7–9 × 2.5–3.0 mm, pubescent abaxially with 2- to 5-branched trichomes, irregularly 7-nerved; *inner tepals* elliptic, obtuse, with membranous inflexed margins, 7–8 × 3 mm wide, pubescent along lower ¾ of midrib abaxially, irregularly 5-nerved; *stamens* as many as tepals, equal, about 5 mm long; *filaments* subulate, ca. 1 mm wide at base, ca. 3.5 mm long; *anthers* 3 mm long, sagittate, retuse at apex; *ovary* obconical, 4–5 × ca. 2–3 mm, villous; *style* ca. 2.8 mm long; *stigma* oblong, obtuse at apex, composed of 3 wide lobes, 0.5 mm long. *Capsule* obovoid, 5–13 × 3–4 mm, sparsely pilose; *seeds* ca. 14 to 19, ovoid, ca. 2 × 1.5 mm, brown; cuticle thick; seed coat bristly with pointed projections.

Distribution and ecology. Congo-Kinshasa and Burundi (Fig. 24), Angola, Malawi.

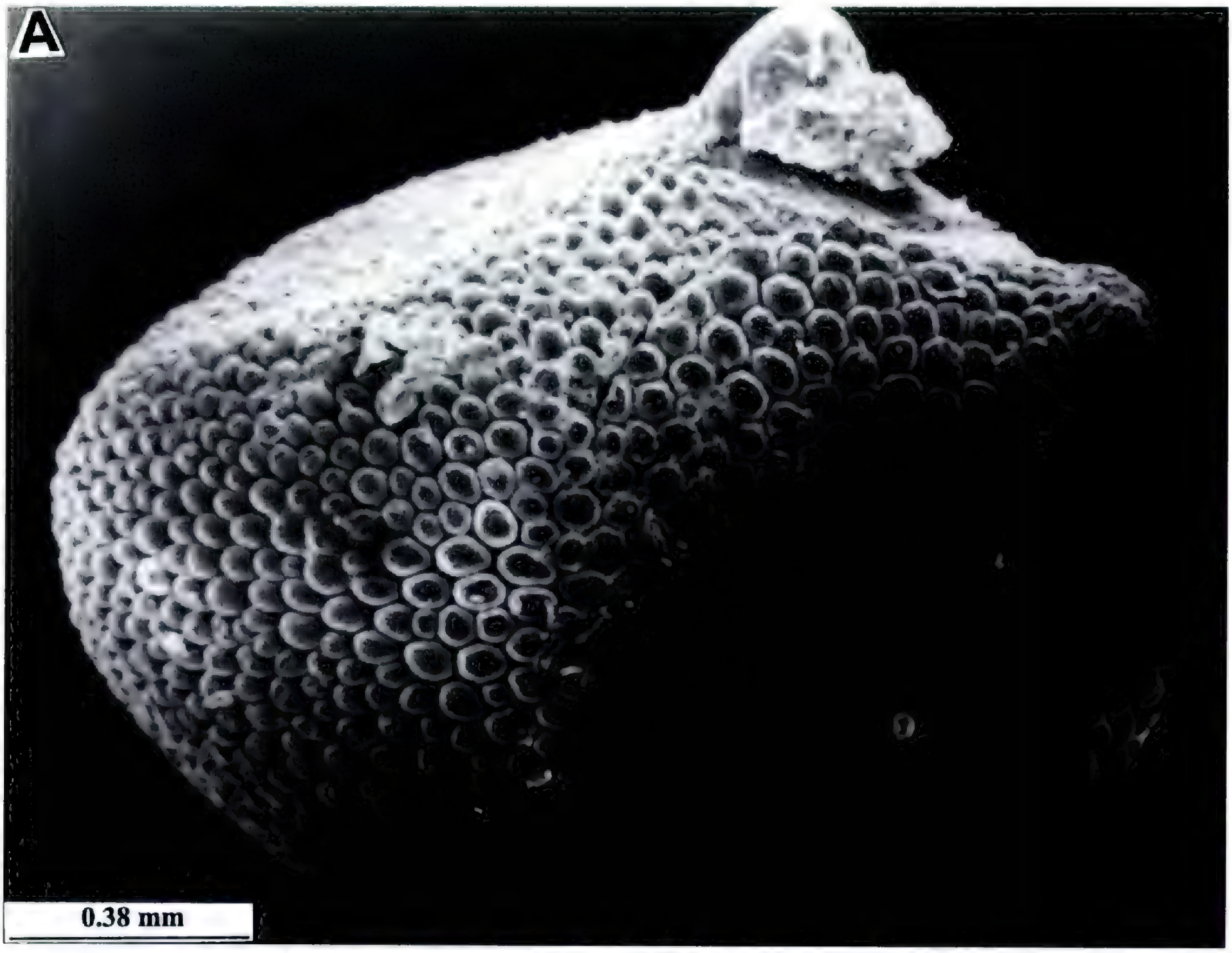
In wet habitats: swamps, on river borders, temporarily inundated grassy savanna, and dembo; alt. 1220–1900 m. Flowering from January to February, in June and October.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Plateau des Kundelungu, rivière Kalunda, *Lisowski, Malaisse & Symoens 12573* (POZG), *Lisowski 81120* (POZG); à env. 10 km au NNW du Poste de Luala, *Lisowski, Malaisse & Symoens 2652* (POZG); environs de Lubumbashi, bord de la Ntwebe, *Lisowski B-7173A* (POZG); P. N. de l'Upemba, piste de la Lufira km 8, *Bamps 851* (BR); Plateau de la Manika, 4 km à W de Katema, *Lisowski, Malaisse & Symoens 184* (POZG); Plateau des Marungu, *Lisowski, Malaisse & Symoens 8268* (POZG); environs de Mulungoshi, Mt. Ngoma, près du village Lusinga, *Lisowski 10710 c* (POZG). BURUNDI. Mosso, *Michel 2534* (BR); Kisozi, *Becquet 2159* (BR).

Hypoxis monanthos was previously reported only from Angola with its range herein extended into Congo-Kinshasa and Burundi (Fig. 24). Most Central African specimens possess single flowers with only 4 tepals. In contrast to many species of *Hypoxis* after flowering, scapes of *H. monanthos* bend to the ground and pedicels bend slightly inward (Fig. 11C). This is an adaptation for seed dispersal in humid habitats and has been noted in other members of the family (Hilliard & Burtt, 1978: 75), for example, *H. decumbens* L. from Central and South America, *Spiloxene aquatica* (L.f.) Fourcade, *Pauridia minuta* (L.f) Durand & Schinz, and *Rhodohypoxis deflexa* Hilliard & Burtt. In Central Africa this character is also found in *H. kilimanjarica*

←

Figure 15. A–C. *Hypoxis malosana*. —A. Habit with fruits. —B. Habit with flowers. —C. Tufted trichome from a scape. —D. *Hypoxis muhilensis* subsp. *muhilensis*, tufted trichome from a scape. A from *Duvigneaud & Timperman 2626* (BRLU); B from *Lisowski 81121* (POZG); C from *Lisowski 81109* (POZG); D from *Lisowski 81116* (POZG).


A
0.38 mm

B
0.05 mm

(Fig. 11A), which also sometimes has flowers with 4 tepals. However, seeds of the two species differ: *H. kilimanjarica* possesses black, colliculate seeds with a thin cuticle (Fig. 12A, B), and *H. monanthos* bears brown seeds with pyramidal projections and a thicker cuticle (Fig. 12C, D). *Hypoxis kilimanjarica* is a montane species that occurs above 1850 m, whereas *H. monanthos* only rarely reaches this altitude. Plants of *H. monanthos* are also similar to another species, *H. filiformis,* but the former has wider leaves to 2–4 mm, whereas the leaves of the latter species do not exceed 0.7 mm in width. Although *H. monanthos* bears seeds similar to those of *H. angustifolia* (Fig. 3), it differs in much shorter pedicels, only to 5 mm long. Pedicels of *H. angustifolia* are at least 12 mm long.

15. Hypoxis muhilensis Wiland, Fragm. Florist. Geobot. 42: 412. 1997. TYPE: Congo-Kinshasa. Shaba: "Domaine de Muhila, au-dessus du village Kalobele," alt. 1690 m, Nov. 1970, *Lisowski 81116* (holotype, POZG!).

Herb to 35 cm high; *rhizome* ovoid or almost spherical, 1.3 × 1.4 cm (dried out); tunic membranous and fibrous, to 7 cm. *Outer leaves* ca. 5, ovate, narrowing toward apex, acute, 2.0–11.5 cm × 6–10 mm, with midrib and margins densely pubescent and with blade surface only sparsely pubescent in apical part below; trichomes 2-branched or tufted; nerves unequal, 11 to 19; *inner leaves* ca. 6, narrowly elliptical or linear, 9.5–35.0 cm × 3–5 mm, densely hispid on midrib and margins beneath, sparsely pubescent above; trichomes 2(3)-branched; nerves unequal, 11 to 19; midrib and margin nerves especially prominent. *Scapes* 4 to 5, 6–20 cm × 1–2 mm, narrowly winged and ciliate in basal half, hispid or pilose apically; *cyme* 3- to 4-flowered; *bracts* subulate, keeled, 6–14 mm long, 1 (3)-nerved, hispid on midrib abaxially; *pedicels* 4–20 mm long, hispid or pilose. *Tepals* 6; *outer tepals* elliptic or lanceolate, 10–13 × 2.5–4.0 mm, densely hispid or pilose beneath, 5- to 7-nerved; *inner tepals* elliptic or ovate, acute, 9–11 × 3.0–3.5 mm, pilose on the lower third or half of midrib abaxially, 5- to 7-nerved; *stamens* equal or unequal; *filaments* subulate; *anthers* linear, sagittate, fused at apex, ca. 3 mm long; *ovary* obovoid, 4–7 × 2–4 mm, densely pilose; *style* ca. 0.5–2 mm long; *stigma* composed of 3 linear lobes, obtuse, or pyramidal, acute, 1.5–2.0 mm long. *Capsule* cylindrical or turbinate, 4–8 × 3–5 mm, hispid or pubescent; *seeds* numerous, ovoid, 1.5–2.0 × 1.0–1.5 mm, red-brown; cuticle thick; seed coat bristly with pointed projections.

Distribution and ecology. *Hypoxis muhilensis* is endemic to Plateau des Muhila in Congo-Kinshasa (Figs. 25, 26). It occurs in grasslands and wooded mountain grasslands; alt. 1400–1690 m. Flowering in November.

Hypoxis muhilensis is similar to *H. angustifolia* due to its loose inflorescences, sparsely pilose linear leaves, and brown seeds. The first possesses, however, a fibrous tunic and is more robust, with leaves thick in texture, solid and rigid pedicels, and dense indumentum on the inflorescences composed of rigid tuft trichomes (Fig. 15D). The second species is characterized by a membranous tunic, leaves thin in texture, slender pedicels, and usually sparse inflorescence indumentum with slender, often only 2–3-branched trichomes (Fig. 18H, I). Moreover, I did not find any collection of *H. angustifolia* from Plateau des Muhila. From *H. canaliculata* and *H. malosana,* two other *Hypoxis* species occurring on this plateau, *H. muhilensis* differs in its brown seeds (Figs. 17, 2C, D) and 3–5 mm wide inner leaves, whereas these two sympatric species possess black seeds (Fig. 16) and narrower leaves 1–3 mm wide (Figs. 6A, 15A). *Hypoxis muhilensis* includes two subspecies.

1a. Outer tepals 12–13 × 4 mm; seed coat cells tightly covered with smooth cuticle, which forms long, filiform projections on their apexes ——— 15a. *H. muhilensis* subsp. *muhilensis*

1b. Outer tepals 10 × 2.5 mm; seed coat cells covered with wrinkled cuticle, pyramidal in outline and short-pointed ——— 15b. *H. muhilensis* subsp. *kansimbensis*

15a. Hypoxis muhilensis subsp. **muhilensis.** Figures. 15D, 17.

Herb to 16 cm high. *Outer leaves* 2–5 × 0.6–1.0 cm; nerves 11 to 17; *inner leaves* 9.5–16.0 cm × 3–5 mm; nerves 17 to 19. *Scapes* 6–9 cm long, hispid apically with tufted trichomes; *pedicels* hispid, 4–15 mm long. *Outer tepals* elliptic, 12–13 × ca. 4 mm, densely hispid abaxially; *inner tepals* narrowly elliptic, ca. 11 × 3.0–3.5 mm, pilose on the lower third of midrib abaxially, 7-nerved; *sta-*

←

Figure 16. *Hypoxis malosana.* —A. Seed. —B. Seed coat sculpture with micropapillae. Both from *Lisowski 81121* (POZG).

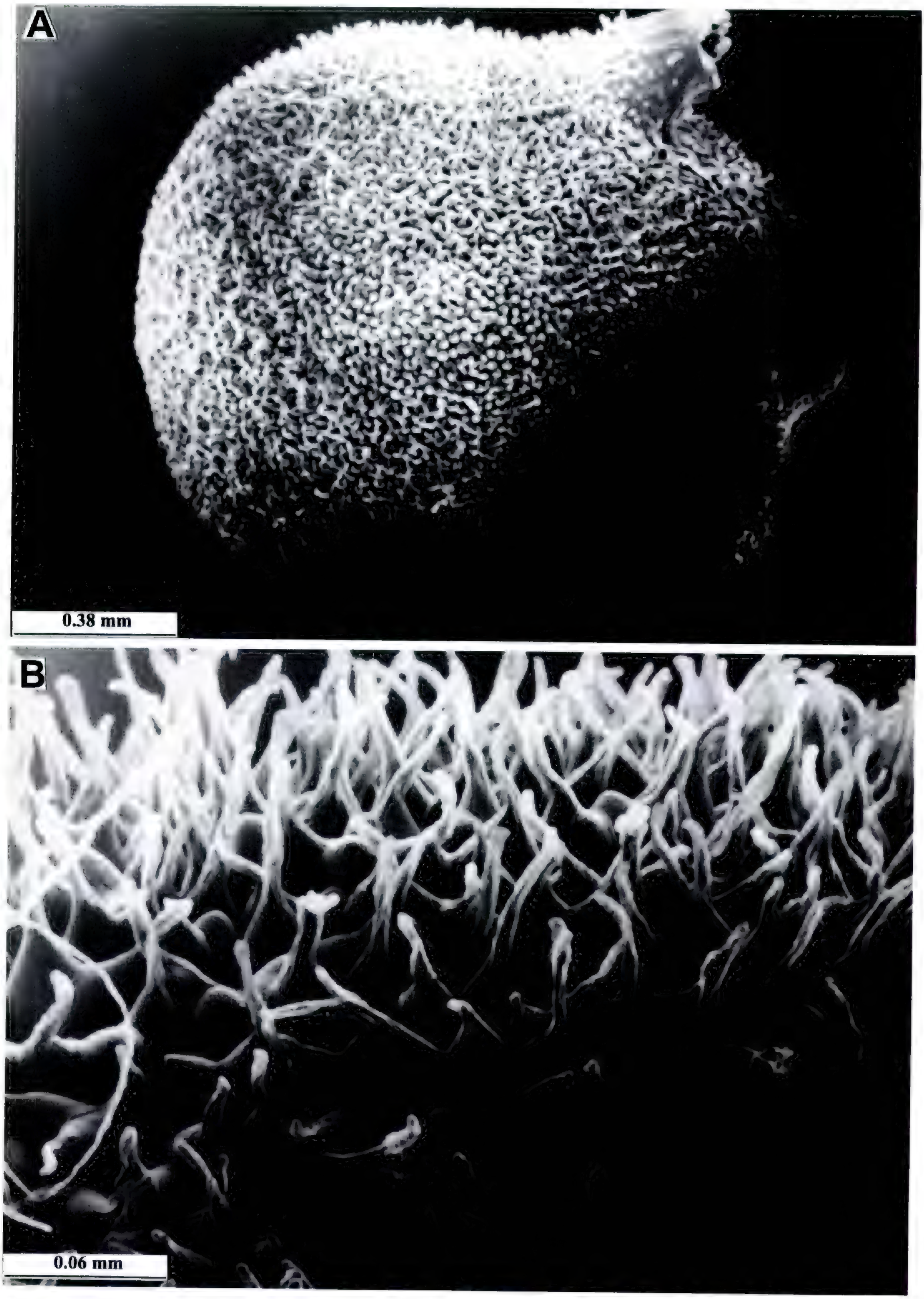


Figure 17. *Hypoxis muhilensis* subsp. *muhilensis*. —A. Seed. —B. Seed coat sculpture. Both from *Lisowski 81134* (POZG).

mens equal, ca. 5.5 mm long; *filaments* subulate, 2.5–3.0 mm long; *ovary* 5–7 × 3–4 mm; *style* ca. 0.5 mm long; *stigma* composed of 3 linear lobes, obtuse, 1.5–2.0 mm long. *Capsule* cylindrical, 4–7 × 4–5 mm, hispid; *seeds* ca. 9; seed coat bristly with long filiform pointed projections.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Domaine de Muhila, au-dessus du village Kansimba, *Lisowski 81134* (POZG).

Hypoxis muhilensis subsp. *muhilensis* differs from all other taxa of *Hypoxis* in unique seed testa sculpture (Fig. 17) with the thick cuticle covering the papillae smoothly, but forming elongated appendages at their apexes.

15b. Hypoxis muhilensis subsp. **kansimbensis** Wiland, Fragm. Florist. Geobot. 42: 414. 1997. TYPE: Congo-Kinshasa. Shaba: Domain de Muhila, près de Kansimba, alt. 1400, Nov. 1970, *Lisowski 81125* (holotype, POZG!). Figure 2C, D.

Herb to 35 cm high; *rhizome* not seen. *Outer leaves* 5.5–11.5 × ca. 1.0 cm, ca. 19-nerved; *inner leaves* linear, 22–35 cm × ca. 3 mm wide, ca. 11-nerved. *Scapes* ca. 4, 9–20 cm long, pilose apically with long white tufted trichomes; *pedicels* 4–20 mm long, pilose. *Outer tepals* lanceolate, 10 × 2.5 mm, pilose abaxially, 5-nerved; *inner tepals* ovate, 9 × 3 mm, pilose on lower half of midrib abaxially, 5-nerved; *stamens* unequal; outer *filaments* ca. 3 mm long, inner ca. 2 mm long; *ovary* 4 × 2 mm; *style* ca. 2 mm long; *stigma* pyramidal, acute, ca. 1.5 mm long. *Capsule* turbinate, 6–8 × 3–5 mm, pubescent, opening by a transverse slit; *seeds* 10–20, ovoid; seed coat bristly with pointed projections.

Although the seeds, characteristically for the species, are covered with thick cuticle, this is folded differently from the other subspecies. In *H. muhilensis* subsp. *kansimbensis* the cuticle is wrinkled and it projects from the papillae so they look like short pointed pyramids (Fig. 2C, D). The cuticle on the papillae in the other subspecies is smooth and creates elongated projections on their apexes (Fig. 17B).

16. Hypoxis robusta Nel, Bot. Jahrb. Syst. 51: 313. 1914. TYPE: Congo-Kinshasa. "Oberer Kongo-Bezirk: Katanga," Oktober 1899, *Verdick 198* (holotype, BR!). Figures 14C, D, 26.

Herb to 80 cm high; *rhizome* subglobose, 3.2–5.2 cm diam. (dried out); tunic black, stiff, 8–12 cm long. *Outer leaves* 3 to 4, sword-like, 5–9 × 3–4 cm, tomentose below, sparsely pubescent above, hispid on edges and midrib abaxially; trichomes tufted, white; nerves unequal, 27 to 29; *inner leaves* ca. 7, narrow-linear, acute, (12.5–) 54–90 × 1.0 (–1.7) cm; indumentum-like on outer leaves, but sometimes also 2-branched trichomes occur; nerves unequal, 15 to 19. *Scapes* 3 to 6, 10–18 cm × 3 (–4) mm, ciliate in lower half, tomentose in apical half; trichomes tufted, white; *raceme* ca. 10-flowered; flower anthesis acropetal; *bracts* 15 × 2 mm, pubescent on the midrib abaxially; *pedicels* 1–10 mm long, tomentose. *Tepals* 6; *outer tepals* lanceolate or narrowly ovate, (12–) 13–17 × 3–5 (–6) mm, abaxially tomentose, irregularly 7- to 11-nerved; *inner tepals* ovate, acute, (11–) 13–14 (–15) × 4–6 (–8) mm wide, irregularly 7- to 9-nerved, pubescent abaxially along midrib; *stamens* equal, 7–14 mm long; *filaments* subulate, fleshy, 2.5–4.5 mm long; *anthers* oblong, prominently sagittate, slightly emarginate at apex, 5–12 mm long; *ovary* obovoid, 5–7 × 4–5 mm, tomentose; *style* often tapering toward base, (1.5–) 2.5–4.0 mm long; *stigma* 2.5–3.0 mm long, composed of three free lobes. *Capsule* obovoid, 5–6 × 4–5 mm, sparsely pubescent; *seeds* numerous, ovoid or almost globose, ca. 1.5 × 1 mm wide, black and glossy; seed coat colliculate with flat papillae.

Distribution and ecology. *Hypoxis robusta* is known only from the Shaba province in Central Africa (Fig. 26). In grassland and wooded grassland, often after burning, on the border of radioactive valley, dembo; sandy soil; alt. ca. 1600 m. Flowering from August to October and in December.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Lukapu, *Verdick s.n.* (BR); Kasompi, *Duvigneaud & Timperman 2175* (BRLU); Kasompi Ouest, *Duvigneaud & Timperman 2209* (BRLU); Kasompi Est, *Duvigneaud & Timperman 2213* (BRLU); sources de la Kamalenge, *Robyns 3891* (BR); Swambo, *Duvigneaud s.n.* (BRLU); territoire de Jadotville [= Likasi], 10 km W. Mindingi, *Duvigneaud & Timperman 2551* (BRLU); Kipopo, *Schmitz 5921* (BR); Plateau de Kundelungu, entre les rivières Petite Lofoi et Kalembe, *Lisowski, Malaisse & Symoens 12984* (POZG).

The flowers on the type specimen of *H. robusta* are very large, in the upper range of variation in this species. Examination of more specimens revealed that the flowers might be smaller on average. However, lanceolate and acute tepals, large anthers to 14 mm long, and deeply divided stigma are very characteristic of *H. robusta.* Because of a similar geographic distribution and leaves covered with white tufted trichomes, this species is similar to *H. hockii.* The two taxa differ in their ecology, because flowers of *H. hockii* are usually accompanied by inner leaves. Flowers are accompanied by outer

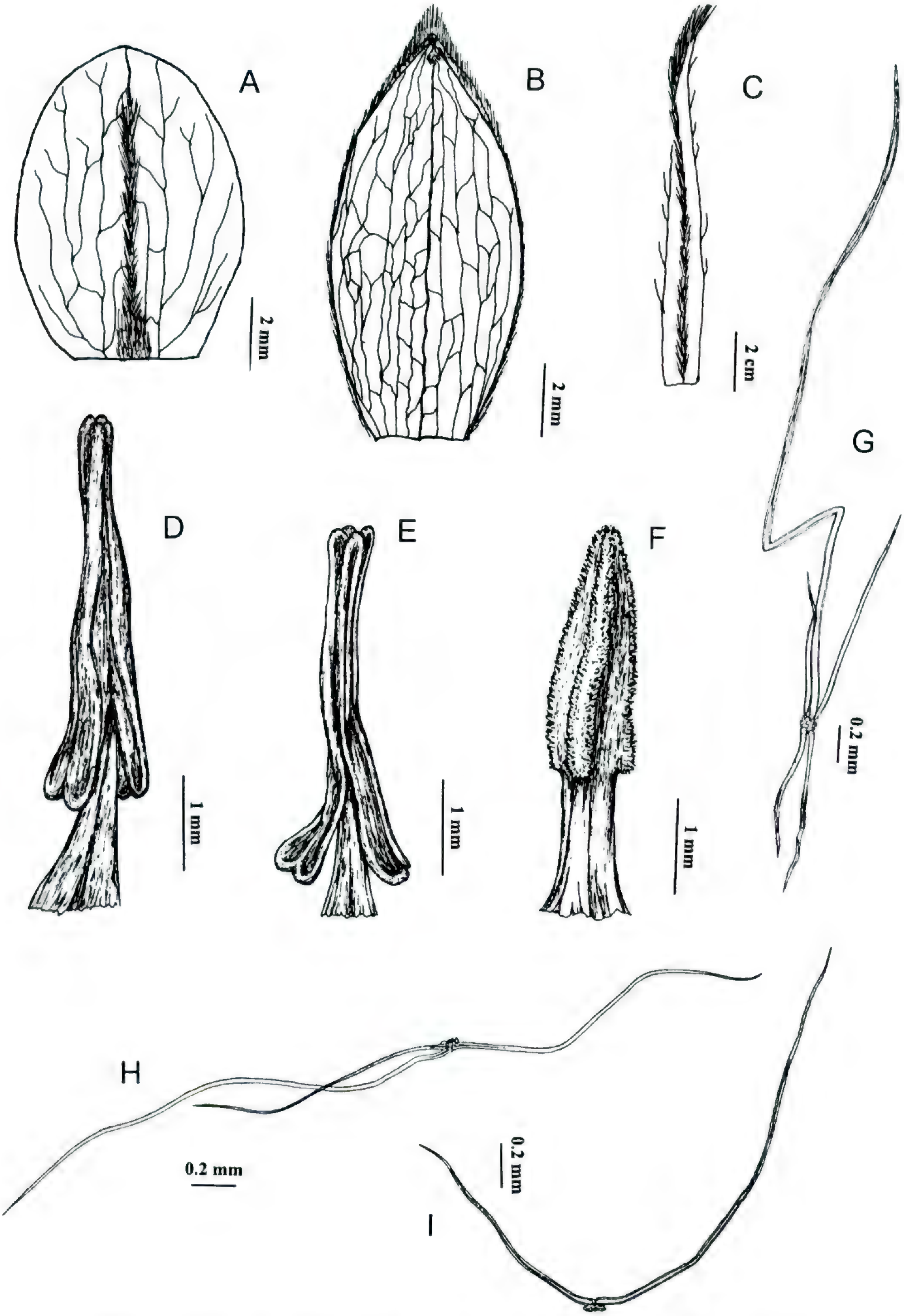


Figure 18. *Hypoxis urceolata.* —A. Dorsal view of inner tepal. —B. Ventral view of outer tepal. —C. Dorsal view of bract. —D. Stamen from the outer whorl (ventral view). —E. Stamen from the inner whorl (ventral view). —F. Pyramidal stigma. —G. Tufted trichome from a scape. H & I. *Hypoxis angustifolia.* Trichomes from scapes. —H. Three-branched trichome. —I. Two-branched trichome. A from *Lejoly 2956* (BRLU); B from *Claessens 1146* (BR); C, D, E, F, G from *Lisowski 48204* (POZG); H from *Lisowski B-7276* (POZG); I from *Lisowski 86119* (POZG).

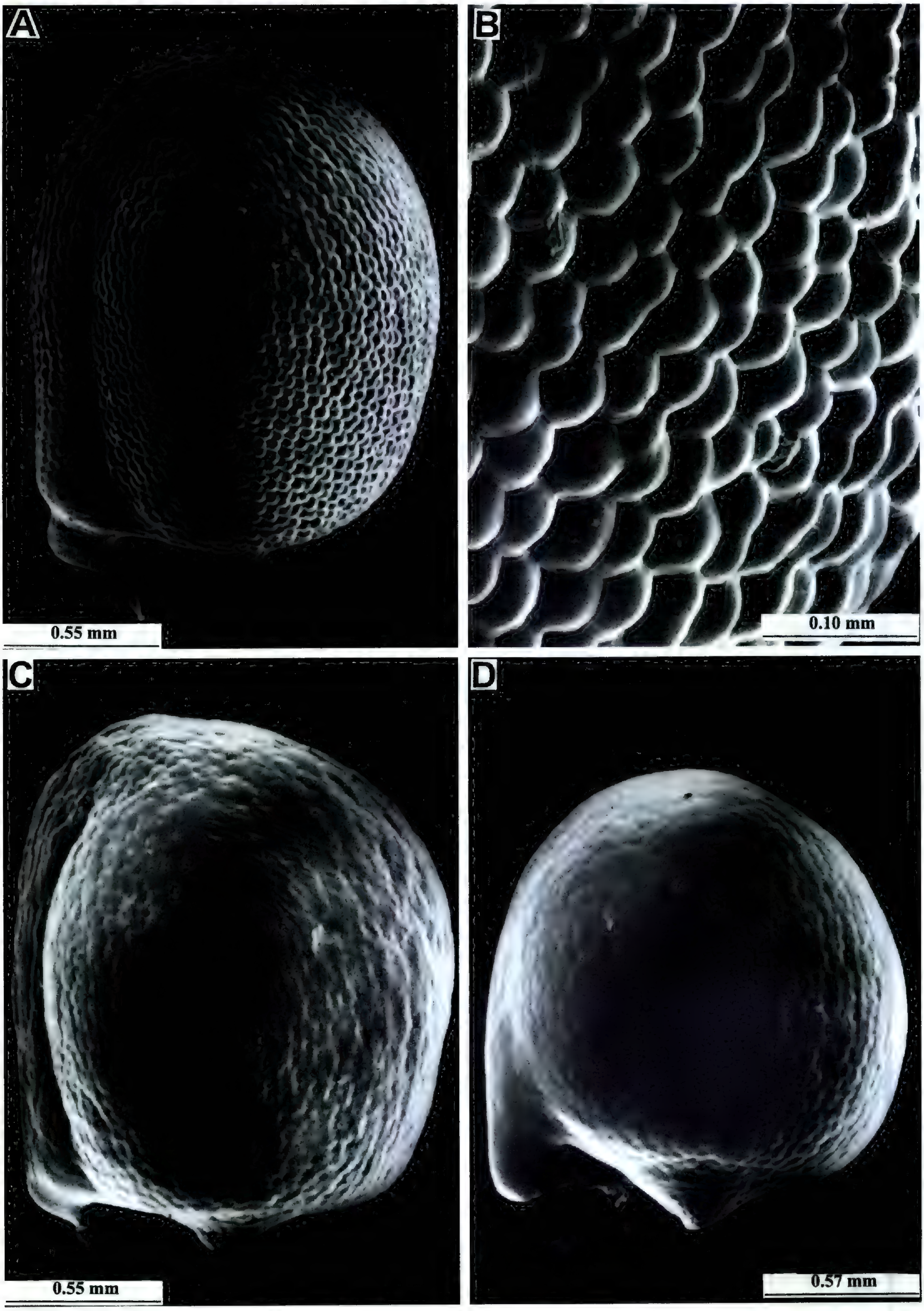


Figure 19. Variability of seeds in *Hypoxis urceolata*. —A, C, D. Seed. —B. Seed coat sculpture. A and B from *Bequaert 4914* (BR); C from *Lebrun 9529* (BR); D from *Lisowski 48204* (POZG).

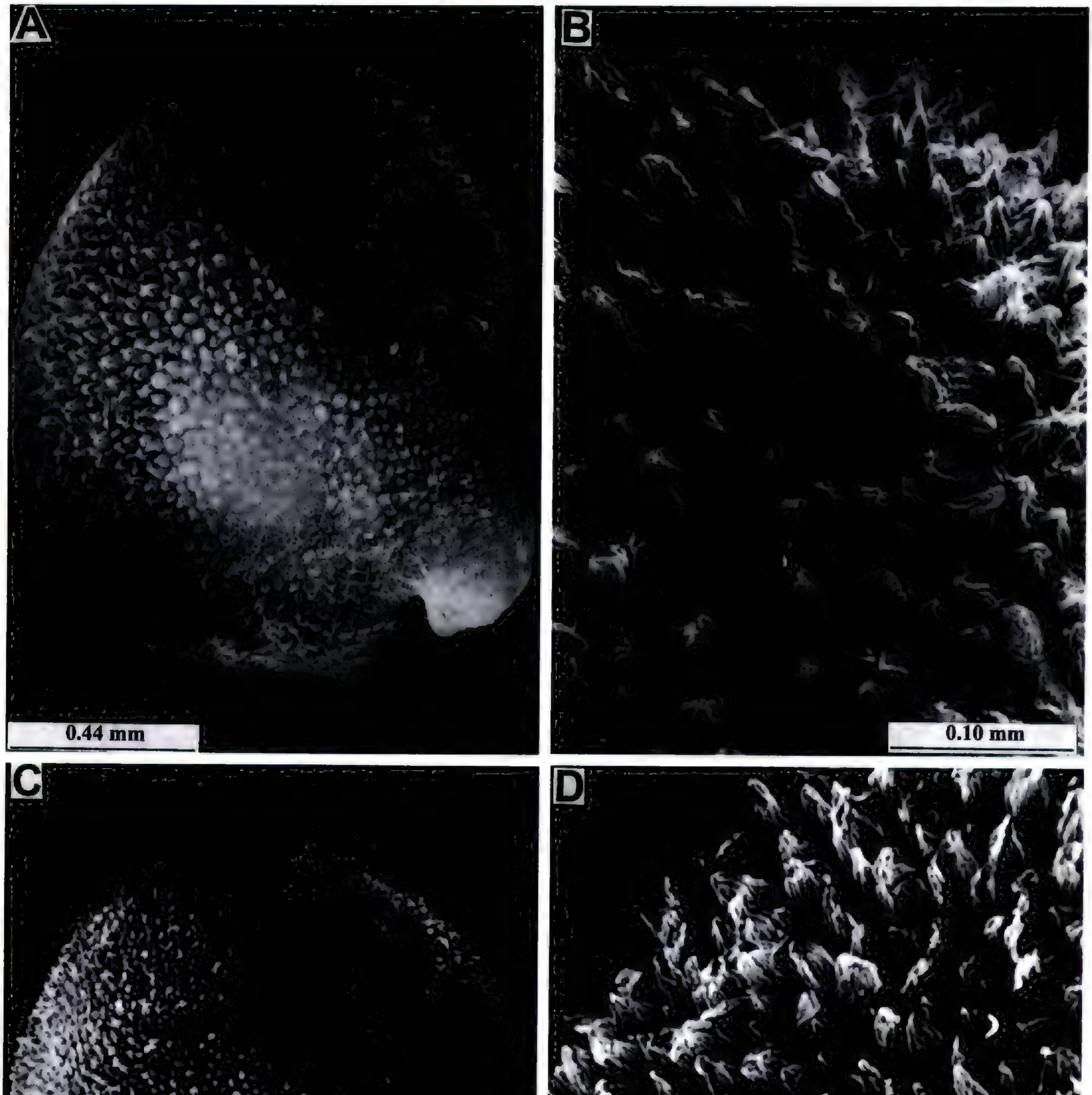


Figure 20. A, B. *Hypoxis symoensiana.* —A. Seed. —B. Seed coat sculpture. C & D. *Hypoxis upembensis.* —C. Seed. —D. Seed coat sculpture. A and B from *Lisowski, Malaisse & Symoens 12058* (POZG); C and D from *de Witte 3698* (BR). Scale for D as in B.

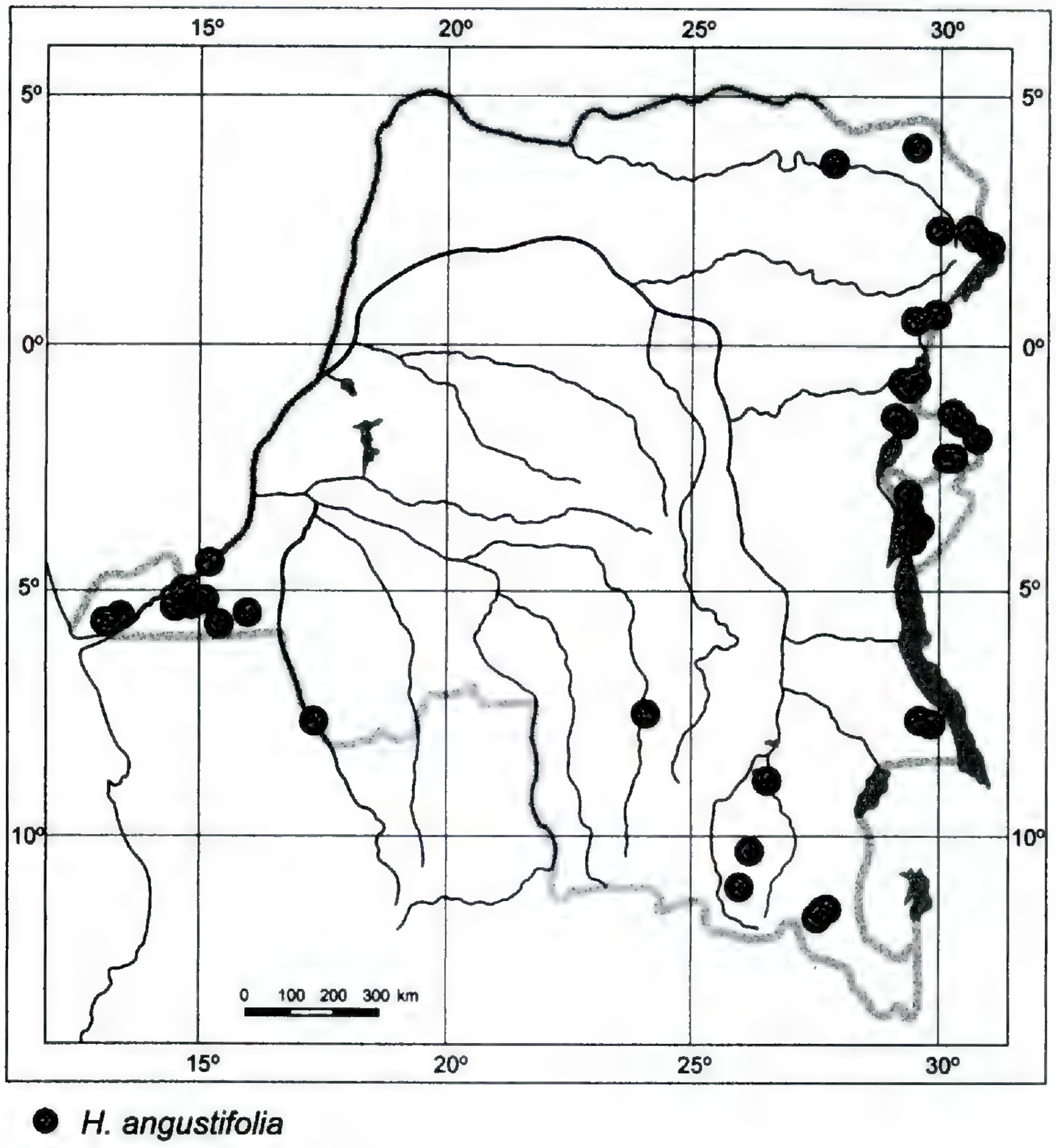


Figure 21. Geographic distribution of *Hypoxis angustifolia* in Central Africa.

leaves only, if any, in *H. robusta*, and its inner leaves do not appear until later. Both species possess black seeds but with different seed coat sculpture (see Figs. 10C–F, 14C, D). The anticlinal cell boundaries in seed coat of *H. robusta* are slightly raised (Fig. 14D), whereas in *H. hockii* var. *colliculata* they are channeled (Fig. 10F). Outer periclinal walls of cells in both taxa are flat (Figs. 10F, 14D), but in the former they are smooth, while in the latter verrucose. Though the anticlinal cell boundaries in two other varieties of *H. hockii* are raised as well, the outer periclinal walls of cells are convex (Fig. 10C, E).

17. Hypoxis subspicata Pax, Bot. Jahrb. Syst. 15: 143. 1893. TYPES: Angola. "Quangogebiet, 10 ½°s. Br.," Sep. 1876, *Pogge 424* (syntype, B!); Malandsche, *Teuscz in von Mechow 249* (syntype, B!). Figure 10A, B.

Herb to 34 cm high; *rhizome* cylindrical, ca. 6 cm diam. (dried out); tunic fibrous, stiff, 6–7 cm high. *Outer leaves* ca. 7, linear, acute, 3.0–6.5 × 1.6–2.0 cm, glabrous basally and above, pubescent below with long, tufted trichomes; nerves unequal ca. 23; *inner leaves* ca. 9, linear-lanceolate, acute, prominently keeled, 20–34 × 0.8–2.0 cm, glabrous above, densely villous below with long white tufted trichomes; nerves of subequal size, closely arranged, ca. 30. *Scapes* 6 or 7, 20–25 cm × ca. 4 mm, narrowly winged and ciliate at base, villous apically with long white tufted trichomes; *cyme* spike-like, 10- to 12-flowered, sometimes branched in apical part and panicle-like; floral anthesis basipetal; the lowermost *bracts* 20–30 × 2–4 mm wide, 5-nerved, pubescent with long trichomes along midrib and on lamina surface in basal and apical parts below, sometimes ciliate; *pedicels* 3–10 mm long, prominently pubescent. *Tepals* 6; *outer tepals* ovate or oblong, 14–15 × ca. 4 mm, densely hispid abaxially, 7- to 9-nerved; *inner tepals* ovate, obtuse, 13–15 × ca. 8 mm, pilose on lower half of midrib abaxially, 7- to 9-nerved; *stamens* equal, ca. 8 mm long; *filaments* subulate, 3.0–3.5 mm long; *anthers* linear, sagittate, fused at apex, 6–7 mm long; *ovary* obconical, ca. 7 × 4 mm, densely pilose; *style* terete, (1.5–) 2–6 mm long; *stigma* composed of 3 linear fused lobes, 2–4 mm long. *Capsule* turbinate, ca. 7 × 5 mm, sparsely white

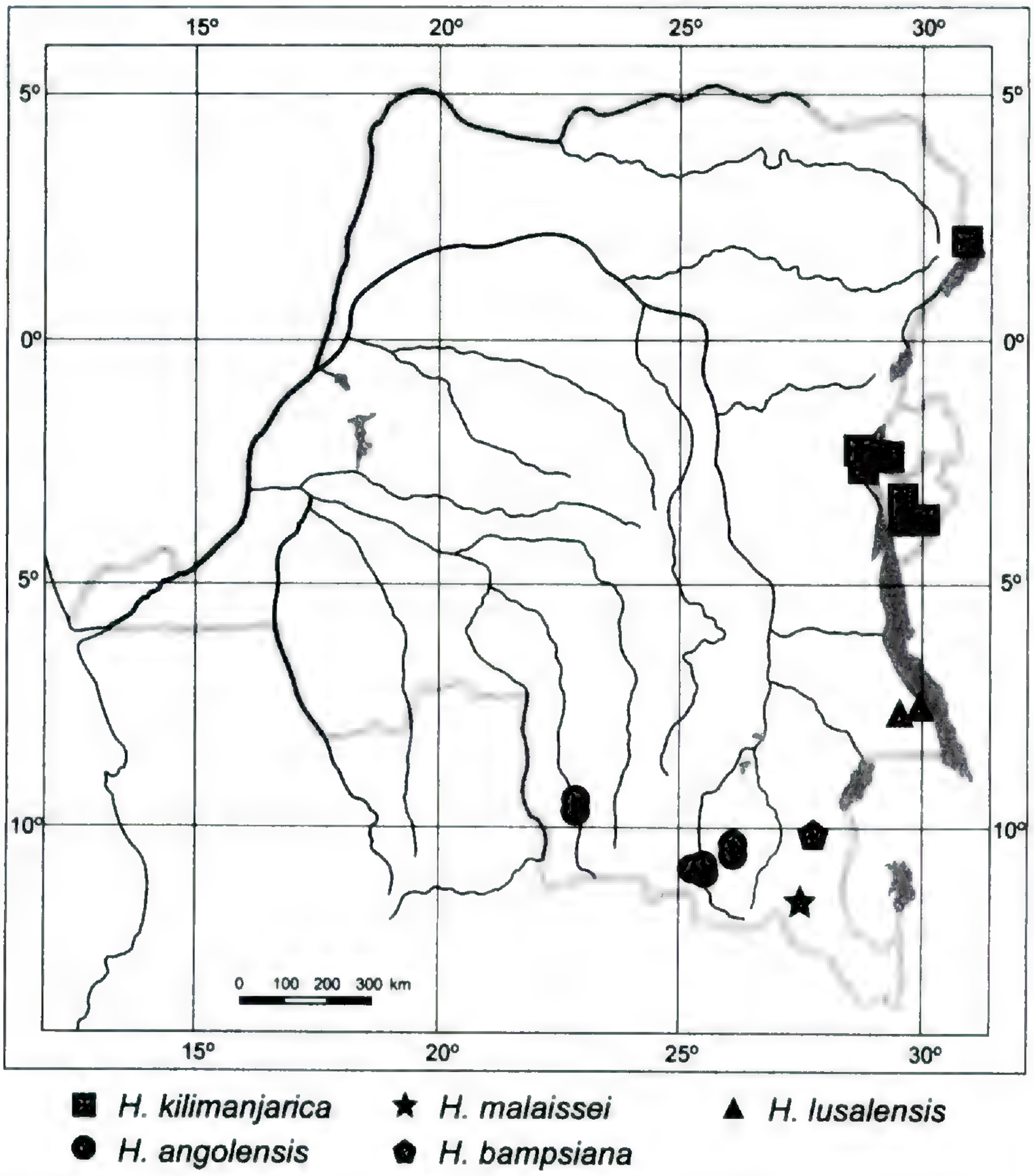


Figure 22. Geographic distribution of *Hypoxis kilimanjarica*, *H. angolensis*, *H. malaissei*, *H. bampsiana*, and *H. lusalensis* in Central Africa.

pubescent; *seeds* ca. 6, almost globular, ca. 1.5 × 1.5 mm, brown; cuticle thick; seed coat tuberculate, with conical papillae covered closely with wrinkled cuticle.

Distribution and ecology. Congo-Kinshasa (Fig. 25), Angola, Zambia. In seasonally dry swamp (dembo); degraded grassland with *Terminalia* and *Diplorhynchus*. Flowering August and November.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Muyeye, *Risopoulos 776* (BR); Kapoka, 28 km E de Kasaji, *Duvigneaud & Timperman 2528* (BRLU); Dikuluwe, *Duvigneaud 4130* (BRLU). ZAMBIA. **Serenje Dist.:** near Serenje, *M. Richards 27606* (MO).

The name *H. subspicata* was used for some time in Central Africa as the name for all species with leaves more than 8 mm wide (Geerinck, 1971). Distribution of *H. subspicata* is very similar to distribution of *H. angolensis*, overlapping across range in Angola and Congo-Kinshasa. Both species share leaves with very densely arranged nerves, but leaves of *H. subspicata* are covered on the entire surface below, whereas leaves of *H. angolensis* are only ciliate on margins and midrib below. These two species have very different inflorescences. *Hypoxis angolensis* possesses racemes with acropetal anthesis, while *H. subspicata* bears cymes with basipetal anthesis. Moreover, seeds of *H. angolensis* are black with thin cuticle, whereas seeds of *H. subspicata* are brown with thicker cuticle. *Hypoxis hockii*, which used to be included in the synonymy of *H. subspicata* (Geerinck, 1971), differs in its acropetal flower anthesis and leaves totally covered with trichomes above and below, and with nerves that are clearly distinct one from another. Moreover, the black seeds of *H. hockii* (Fig. 10) contrast it with *H. subspicata*. Seeds of the latter species are covered with thick wrinkled cuticle (Fig. 10A, B), while seeds of *H. hockii* have thin cuticle (Fig. 10C–F), and in two varieties papillae are of different shape: spiny in *H. hockii* var. *katangensis* (Fig. 10E) and flat in *H. hockii* var. *colliculata* (Fig. 10F).

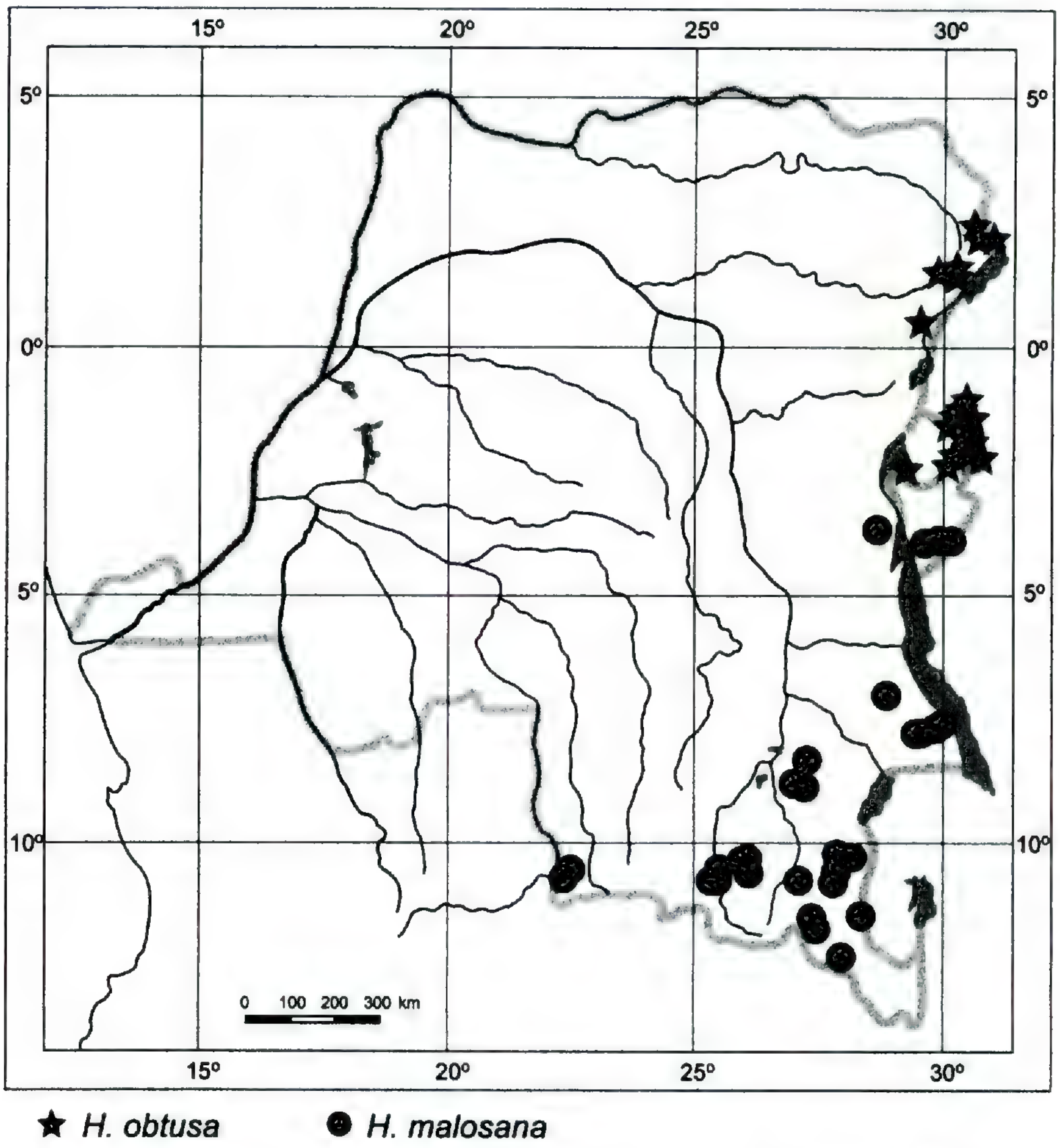


Figure 23. Geographic distribution of *Hypoxis obtusa* and *H. malosana* in Central Africa.

18. Hypoxis symoensiana Wiland, Fragm. Florist. Geobot. 42: 421–422. 1997. TYPE: Congo-Kinshasa. Shaba: "Plateau des Marungu, environ de Kasiki, près de Mare Kasozia," alt. 2050 m, Nov. 1970, *Lisowski, Symoens & Malaisse 12058* (holotype, POZG!). Figures 20A, B, 6B.

Herb to 7.5–10 cm high; *rhizome* ca. 2.5 cm diam. (dried out); tunic fibrous, 3.0–5.5 cm high. *Outer leaves* ca. 3, 3.3–5.5 × 0.8–1.2 cm, ovate, ciliate on margins and midrib below; nerves unequal, ca. 21; *inner leaves* narrow-linear, 4.5–10.0 cm × 2–3 mm, long hispid on margins and midrib below; nerves unequal, ca. 9. *Scapes* 4 to 5, 4.5–9.5 cm × ca. 2 mm broad just below inflorescence, narrowly winged and ciliate at base, pubescent; raceme 2- to 4-flowered; *bracts* single, rarely 2, very narrow, 5–10 mm long, pubescent along midrib abaxially; *pedicels* 3–8 mm long, pubescent. *Tepals* 6; *outer tepals* ovate, acute, ca. 7 × 2.5 mm, pubescent abaxially, irregularly 7-nerved; *inner tepals* ovate, obtuse at apex, ca. 7 × 3 mm, pubescent along lower half of midrib adaxially, irregularly 7-nerved; *stamens* equal, ca. 4 mm long; *filaments* ca. 2.5 mm long; *anthers* ca. 3 mm, sagittate, fused at apex; *ovary* obconical, ca. 3 × 2.5 mm, pubescent; *style* subulate, 1.5 mm long; *stigma* composed of three oblong lobes, emarginate at apex, ca. 1.2 mm long. *Capsule* obovoid, ca. 5 × 4 mm, pubescent, opening apically by a transverse slit; *seeds* numerous, ovoid, ca. 1.8 × 1.2 mm, red-brown; cuticle thick; seed coat bristly with pointed projections.

Distribution and ecology. A species known only from three high elevated plateaus in Shaba in Congo-Kinshasa (Fig. 26), where it occurs in grasslands and miombo; alt. 1650–2050 m. Flowering from September to October.

Additional specimens examined. CONGO-KINSHASA. **Shaba:** Plateau des Kundelungu, 5 km to the S of W source of the Lutshipuka river, *Malaisse 6042* (POZG); Plateau de la Manika, Kolwezi, *Plancke 167/2613* (BRLU).

Small in size and with hispid indumentum, *Hypoxis symoensiana* is distinguished from other small *Hypoxis* species by its racemose inflorescence (Fig. 6B). It is similar in appearance to *H. canaliculata* (Fig. 6A) because of size and light coloring of indumentum. *Hypoxis symoensiana* differs in its brown seeds covered with thick cuticle as seen in

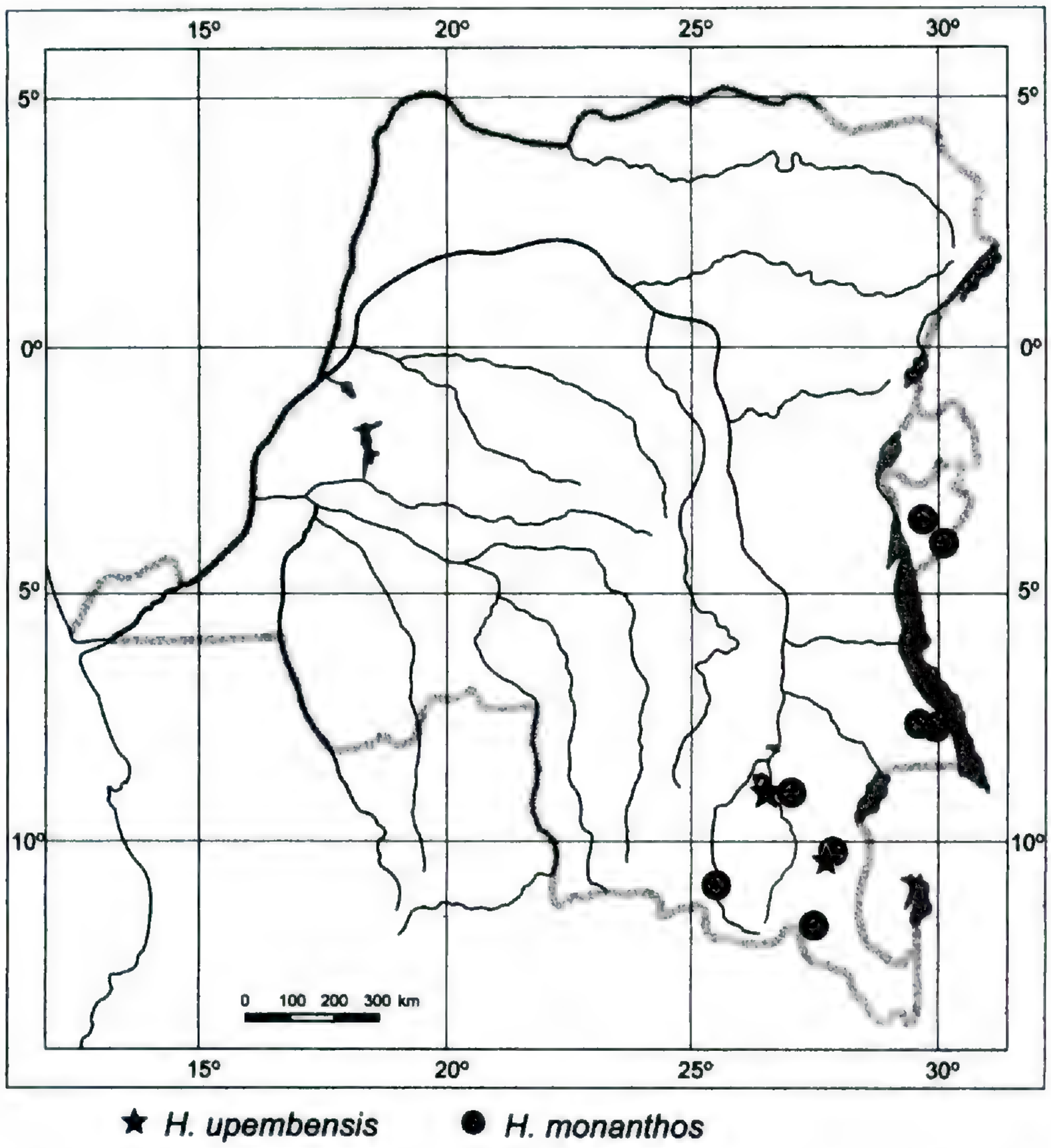


Figure 24. Geographic distribution of *Hypoxis monanthos* and *H. upembensis* in Central Africa.

Figure 20A, B. Seeds are not black with a thinner cuticle, as in the latter species.

19. Hypoxis upembensis Wiland, Fragm. Florist. Geobot. 42: 414. 1997. TYPE: Congo-Kinashasa. Shaba: "Parc National Upemba, riv. Katongo, affluent of Kubale, savane herbeuse, 1750 m," *de Witte 3698* (holotype, BR!). Figures 6E, 20C, D.

Herb to 21 cm high; *rhizome* globose, 15 mm diam. (dried out), white inside; tunic membranous and fibrous, to ca. 5 cm. *Leaves* 5 to 10, subulate, linear, 7–21 cm × ca. 2 mm, with membranous margins in the basal part, ca. 9-nerved; young leaves sparsely pilose on margins and midrib below with 2-branched trichomes; old leaves glabrous. *Scapes* 3 to 4, 11.5–21.0 cm × ca. 1 mm, in basal part very narrowly winged and glabrous or with several trichomes, in upper half sparsely pilose with 2-branched trichomes; *inflorescence* 2-flowered; *bracts* distinctly wider in basal part, ca. 11 × 1 mm, 1-nerved, sparsely pilose on the midrib abaxially with simple or 2-branched trichomes; *pedicels* to 6 mm long, pilose with tufted trichomes. *Tepals* 6; *outer tepals* elliptic, ca. 10 × 5 mm, 7-nerved, abaxially pilose; trichomes golden yellow, 2-branched and tufted; *inner tepals* oblong or ovate, acute or obtuse at apex, 9–10 × ca. 4 mm, abaxially pilose at base, 7-nerved; *stamens* equal, ca. 5 mm long; *filaments* ca. 3 mm long; *anthers* linear, ca. 3 mm long, deeply sagittate, fused at apex; *ovary* obconical, 5–7 × ca. 3 mm, pubescent; *style* stout, ca. 2.5 mm long; *stigma* capitate, composed of 3 oblong lobes, ca. 1 mm long. *Capsule* obconical, ca. 6 × 3 mm, pubescent; *seeds* few, irregularly globose, ca. 1.5 × 1.5 mm, brown; cuticle thick; seed coat bristly with pointed projections.

Distribution and ecology. Known only from Shaba in Congo-Kinshasa (Fig. 24), where it occurs in grasslands; alt. 1750 m. Flowering in April.

Additional specimen examined. CONGO-KINSHASA. **Shaba:** Kundelungu Plateau, between the source of Lofoi and Katschupa, *Hooper & Townsend 532* (K).

Vernacular name. Kadjidji, Kabu (dialect Kiluba).

Hypoxis upembensis is a distinctive taxon with its two flowers clustered together and comparatively

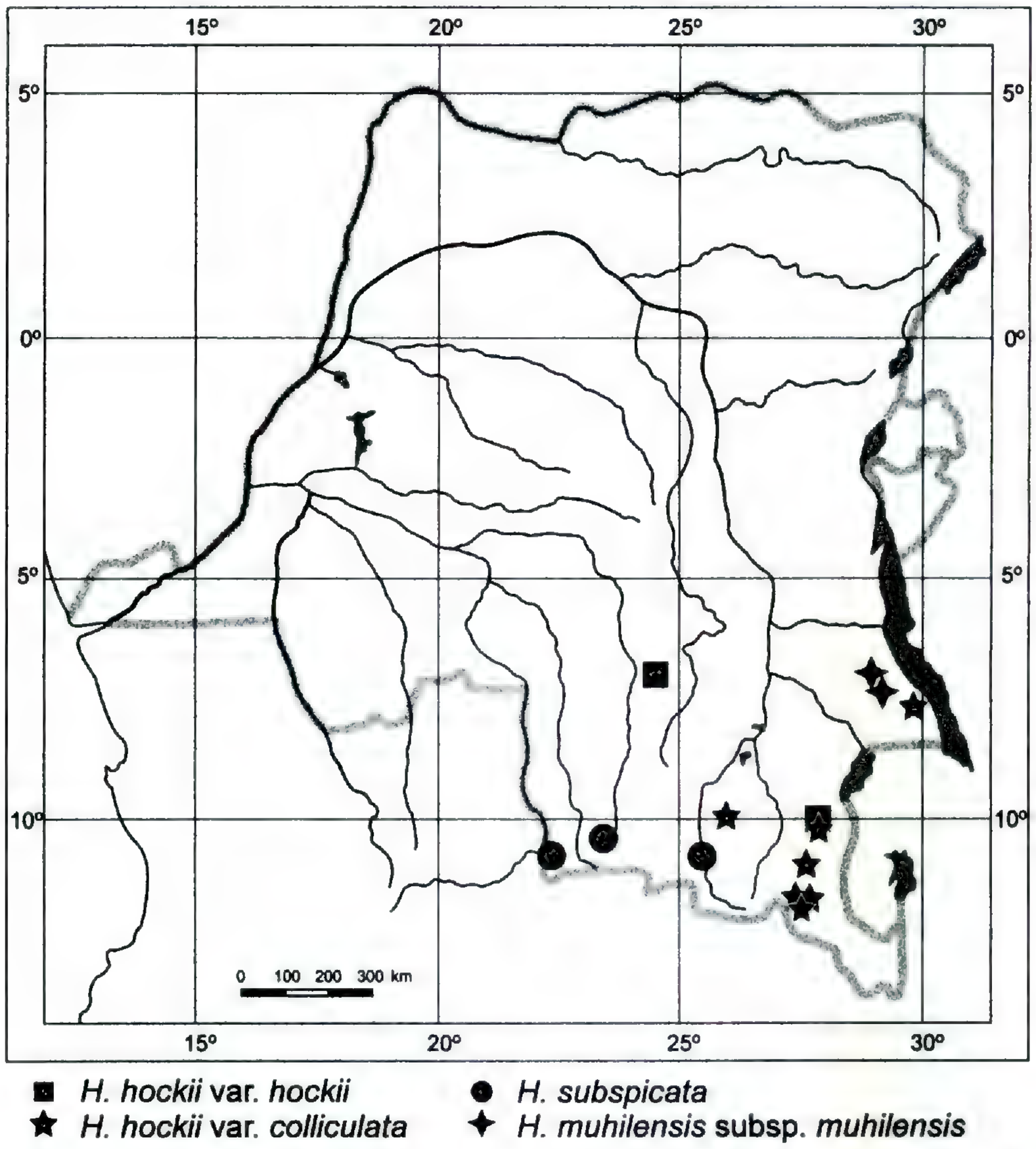


Figure 25. Geographic distribution of *Hypoxis hockii* var. *hockii*, *H. hockii* var. *colliculata*, *H. subspicata*, and *H. muhilensis* subsp. *muhilensis* in Central Africa.

wide tepals (Fig. 6E). Its rhizome is edible (*de Witte 3698*). With *H. angustifolia* this taxon shares similar sparse indumentum composed of 2-branched trichomes and seeds covered with thick cuticle (Figs. 3, 20C, D). The papillae of *H. upembensis* seem to be more elongated than in the former species. *Hypoxis upembensis* is easy to distinguish by its partially fibrous tunic, outer tepals to 5 mm, and shorter pedicels to 6 mm in length. The tunic of *H. angustifolia* is entirely membranous, its tepals are shorter and do not exceed 4 mm in width, but its pedicels are longer, usually to at least 12 mm.

20. Hypoxis urceolata Nel, Bot. Jahrb. Syst. 51: 336. 1914. TYPE: Tanzania. Sine loc., *Obst s.n.* (syntype, B!). Figures 18A–G, 19.

Hypoxis crispa Nel, Bot. Jahrb. Syst. 51: 334. 1914. TYPE: Tanzania. "Kilimandscharo, Landschaft des Ngowe (Muengue), auf einem kahlen mit Grass bedeckten Sandhügel, um 1550 m ü. M., Juni 1893," *Volkens 360* (holotype, B!).

Hypoxis apiculata Nel, Bot. Jahrb. Syst. 51: 327. 1914. TYPE: Tanzania. "Kilimandscharo, auf dem Gipfel, N'di (Taita) Berg, February, 1877," *Hildebrandt 2542* (holotype, B!).

Hypoxis bequaertii De Wild., Pl. bequaerti. 1: 49. 1921. TYPE: Congo-Kinshasa. Haut-Zaïre: "entre Irumu et Bogoro, savane herbeuse, fleurs jeunes intérieurement, vertes extérieurement, 7 juillet 1914," *Bequaert 4919* (syntype, BR!). Kivu: entre Beni et Kasindi, steppe à *Acacia*, fleurs jaunes, 8 août 1914, *Bequaert 5198* (syntype, BR!).

Herb to 60 cm high; *rhizome* ovoid, 3.3–6.5 × 2.3–3.0 (4.3) cm (dried out), yellow, orange, or brown inside with yellow sap; tunic fibrous, to 10 cm long. *Outer leaves* 1 to 5, oblong-ovate, (4.5–) 6–13 × 0.8–2.0 (–2.4) cm, sometimes reflexed, pubescent; trichomes on margins and midrib abaxially tufted, on lamina surface 2-branched; nerves unequal, 17 to 28 (to 33); *inner leaves* 5 to 15, linear, (11–) 17–60 (–95) × 0.8–2.0 cm, sometimes reflexed; indumentum like above, with trichomes falling off with age; nerves unequal, (12–) 19 to 45. *Scapes* 4 to 9, 8–45 cm × 2–3 mm, ciliate in lower half, pubescent above; trichomes tufted, ca. 5-branched, golden; *raceme* 2- to 7-flowered; *bracts* subulate, keeled, (5–) 9–24 × 1–2 mm, 3-nerved at the lowest flowers, pubescent on midrib abaxially, ciliate on margins; *pedicels* 0 or 3–35 mm long,

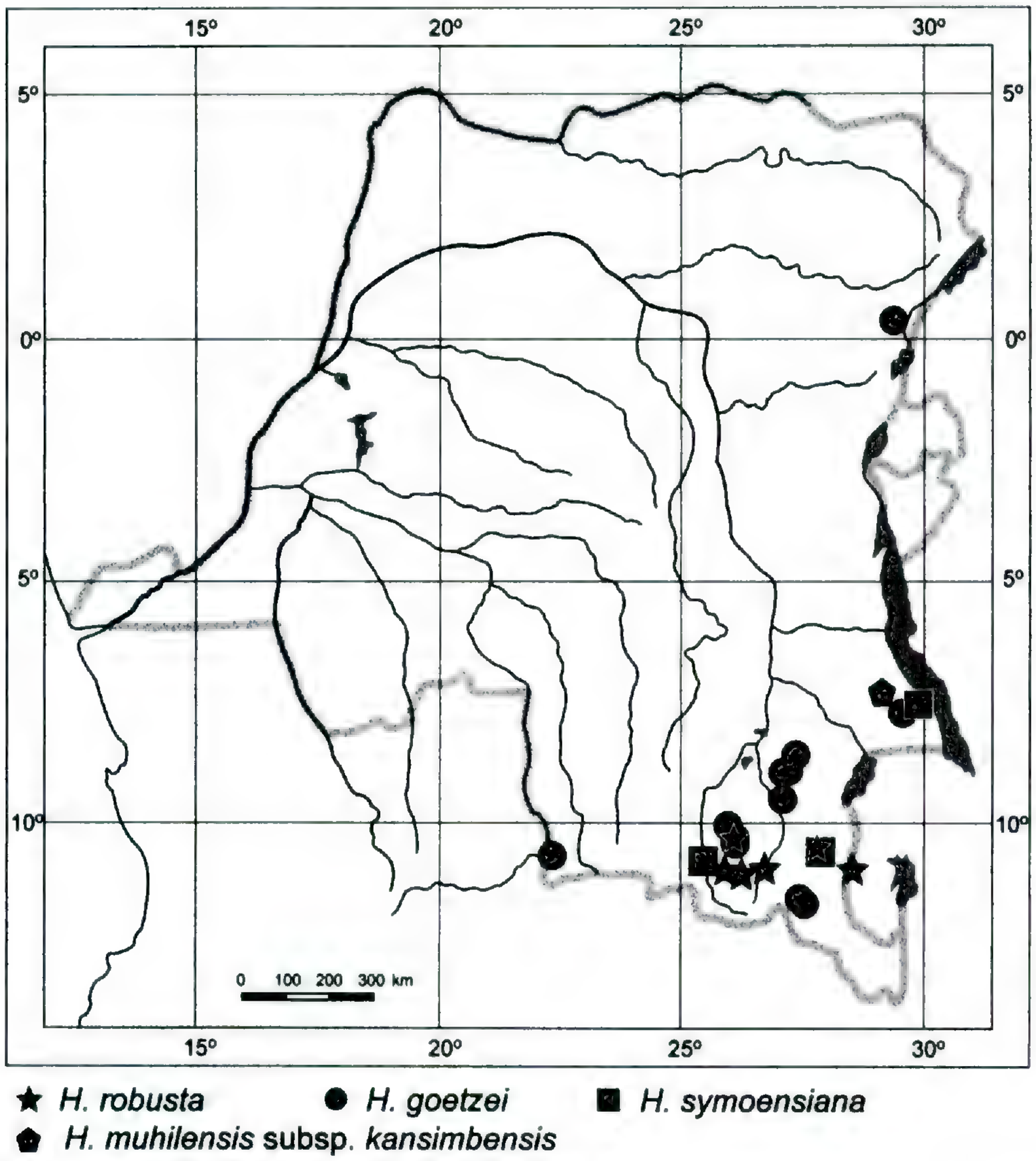


Figure 26. Geographic distribution of *Hypoxis robusta*, *H. goetzei*, *H. symoensiana*, and *H. muhilensis* subsp. *kansimbensis* in Central Africa.

tomentose. *Tepals* 6, all yellow or outer green; *outer tepals* ovate, 8–15 × 4–5 (–6) mm, keeled, pubescent abaxially, irregularly 5- to 7-nerved; *inner tepals* ovate, obtuse, 8–14 × ca. 5 mm, irregularly 5- to 7-nerved, pubescent abaxially along the midrib in lower ¾; *stamens* unequal; outer stamens 4–8 mm long; inner stamens 4–7 mm long; *filaments* subulate, 1.5–4.0 mm long; *anthers* oblong, prominently sagittate, fused at apex, 3–6 mm long; *ovary* obconical, 4–10 × 3–4 mm, villous; *style* 3–4 mm long; *stigma* pyramidal, 2–3 mm long, with 3 stripes of papillae. *Capsule* turbinate, 4–10 × 3–5 mm, pubescent; *seeds* numerous, ovoid, ca. 1.5 × 1 mm, black; seed coat colliculate with ± flat papillae.

Distribution and ecology. Probably all of eastern Africa. In Central Africa in northeastern parts of Congo-Kinshasa and in Rwanda. Occurs in grasslands with xeromorphic shrubs or woodlands; at termite mounds; in herbaceous fallow fields, degraded pasture; in wet areas, water flows, on rocky, kaolin soil; alt. 1200–2000 m. Flowering all months except June and November. Inflorescences are accompanied by leaves.

Additional specimens examined. CONGO-KINSHASA. **Haut-Zaïre:** Mahagi, *Lebrun 3755* (BR); Nioka, *Germain 3962* (BR), *Wilbaux 167* (BR), *Menager 67* (BR); S of Golu, N of Nioka, *Sperry 353* (BR); Bunia, *Lejoly 2949 A* (BRLU) & *2956* (BRLU, BR), *Claessens 1146* (BR), *Lisowski 48204* (POZG); Kilo Moto, *Foscolos 19* (BR); Bogoro–Geti-Road, *Johnston 1117* (BR). RWANDA. S. l., *Burgeon 14* (BR); Mugariro, *Christiaensen 1553* (BR); Parc National de la Kagera, Kibungu, S de Ndama, *Robyns 3553* (BR); colline Muhororo, *Troupin 8864* (BR); plaine d'Uruwita, *Troupin 5287* (BR); Byumba, colline Rwisirabo, *Troupin 13969* (BR); colline Kizirakome, *Troupin 8606* (BR); Gabiro, *Germain 2854* (BR); environs de Gabiro, *Bouxin & Radoux 1105* (BR); colline Nyarutovu, *Troupin 14108* (BR); piste Gabiro—Kakitumba km 18, *Van der Veken 10754* (BR); Biumba, *Lebrun 9529* (BR), *Troupin 2699* (BR); territoire Biumba, Kijojo, *Christiaensen 800* (BR); Nyakatare route vers Gabiro, *Taton 957* (BR); Rusumo—Kibungo km 13, *Bamps 2727* (BR); Rwinkwavu, *Bouxin & Radoux 1244* (BR); région du Bugesera, colline Biharagu, *Troupin 9474* (BR); Bulenge, *Van Oosten 106* (BR), *Liben 657* (BR); Gashora, *Augier 2888* (BR); Lac Mohasi, *Becquet 590* (BR); environs Mimuli, colline Bi-

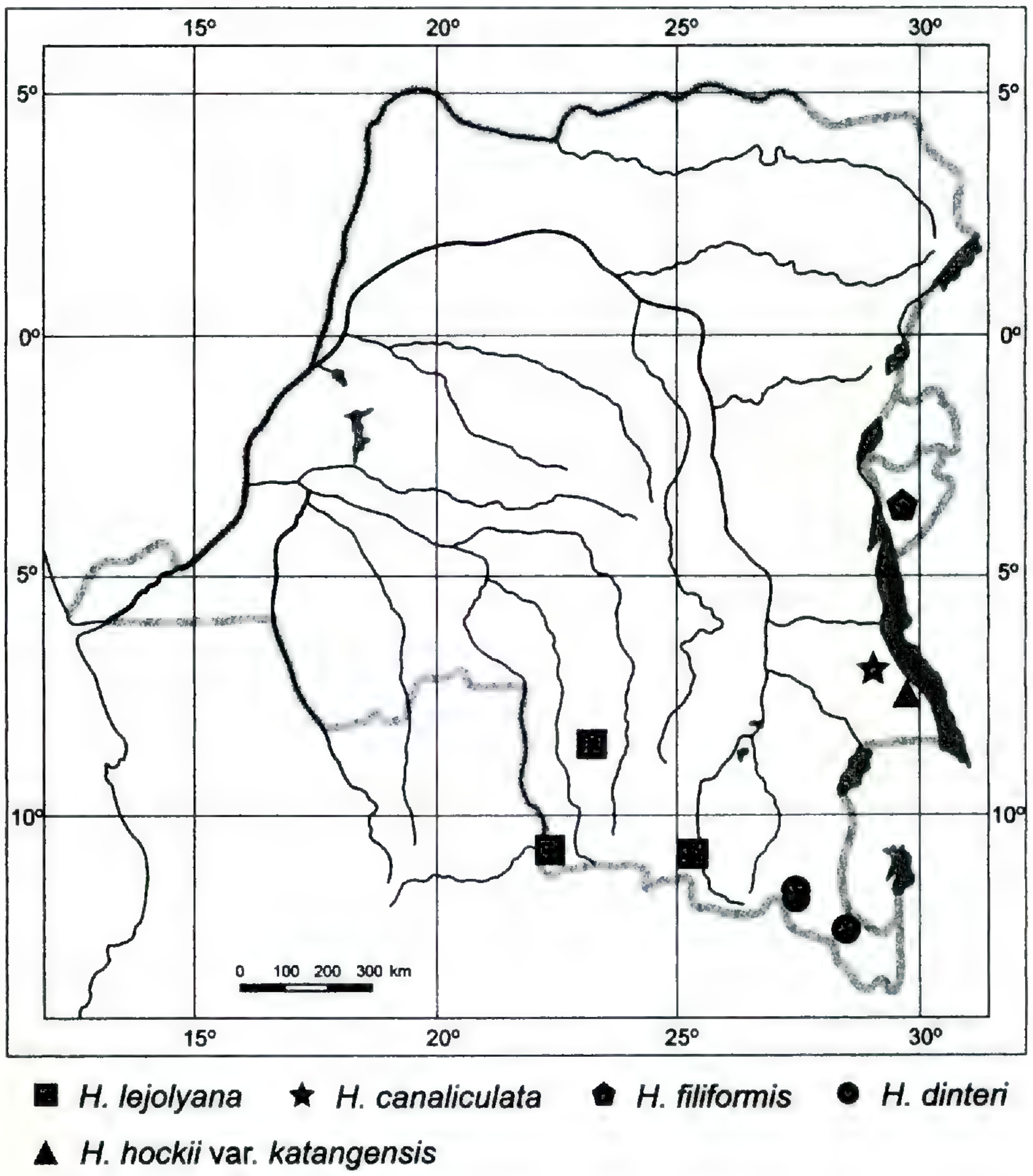


Figure 27. Geographic distribution of *Hypoxis lejolyana, H. canaliculata, H. filiformis, H. dinteri,* and *H. hockii* var. *katangensis* in Central Africa.

bale, *Troupin 4800* (BR); Région du Mutara, route Kigali—-Kakitumba vers km 195, *Troupin 8199* (BR).

Vernacular name. Moleke (dialect Kilendu); Adingtong, Ucongo (dialect Kilur); Milia-baniki (dialect Babira); Mutjutjuka, Nyaboshya, Nyabohya, Nyabohye, Nyabokyera, Wawazotahili (Kinyaruanda); Isheshe (Rwanda, *Becquet 590*).

The rhizome of *Hypoxis urceolata* is boiled, and the extract is used as a first purgative for new born babies. *Hypoxis urceolata* forms a distinct group in Central Africa because of its distribution in the northern part of the region. This species is, however, very variable in its morphology. Plants differ in leaf width and recurvation. Their indumentum might be of varying density as well. However, stamens are usually fused at the apex (Fig. 18D, E) and the stigma is pyramidal in shape (Fig. 18F). There is no agreement as to what are the proper name and delimitation of this complex taxon in other parts of Africa (see Nordal et al., 1985). Some authors (e.g., Zimudzi, 1996; Nordal, 1997) prefer to use the name *H. villosa* L., which indeed has priority, but because there is no clear delimitation of the complex, different authors include different entities in its synonymy. I saw only microfiche from LINN with the type of *H. villosa* and South African specimens of this species, but I am convinced that this is not the same species as occurs in Central Africa, because of a different type of inflorescence. *Hypoxis urceolata* possesses a rather dense raceme, while *H. villosa* has a lax corymb. The situation is complicated by proven apomixis in the *H. urceolata* complex, which is also called the *H. obtusa* complex (Nordal et al., 1985; Zimudzi, 1994). *Hypoxis obtusa* is a very distinct South African species with recurved leaves, among Central African species similar in texture to leaves of *H. angolensis,* with approximate nerves and ciliated only on the margins and midrib. Leaves of *H. urceolata* are totally covered with indumentum, and veins are distant one from another. Another name perhaps applicable to the Central African plants is *H. hemerocallidea* Fisch. & Mey. as proposed by Hilliard and Burtt (1983) for a group of South African plants

included previously in *H. obtusa* or *H. villosa*. I did not see, however, a type specimen of this species, and because nomenclature applied to this group is one of the most complicated in the whole genus, further studies including materials from South Africa need to be performed to solve this problem. For the purpose of this publication, I therefore decided to use the oldest application of the name for this species in Central Africa, namely *H. urceolata* Nel, which was previously reported from Rwanda (Troupin, 1971; Champluvier, 1987).

Hypoxis urceolata is allopatric in Central Africa to all species with leaves of similar size, namely *H. robusta, H. subspicata, H. angolensis*, and *H. hockii*. A character that usually occurs in the former species, but in none of the others, is a ciliated bract (Fig. 18C). Seeds of *H. urceolata* are always black and glossy and with thin cuticle. The anticlinal cell boundaries are slightly raised (Fig. 19B); outer periclinal walls of cells may be flat (Fig. 19C, D) or concave (Fig. 19A, B), another polymorphic character in this taxon. From *H. subspicata*, the discussed species differs in its seeds (Fig. 19), inner leaves totally covered with indumentum and with distant nerves, and no more than 7 flowers in the racemes. The latter species possesses brown seeds with thick cuticle (Fig. 10A, B), inner leaves glabrous above and with approximate nerves, and 10 to 12 flowers in cymes. The seeds of *H. robusta* are very similar in seed testa sculpture to seeds of *H. urceolata* (Fig. 14C, D). However, the two species differ not only in geographical distribution but also in phenology. *Hypoxis robusta* bears about 10-flowered inflorescences long before leaves, whereas in the latter species they occur simultaneously with leaves producing no more than 7 flowers each. In contrast to *H. urceolata, H. angolensis* possesses seeds with the anticlinal cell boundaries slightly channeled (Fig. 2B) and leaves ciliate on midrib and margins below only (Fig. 1A) and with approximate nerves. *Hypoxis hockii* is distinguished by seeds with the raised convex papillae in *H. hockii* subsp. *hockii* (Fig. 10C) and *H. hockii* subsp. *katangensis* (Fig. 10E), as well as flat but micropapillated papillae with channeled anticlinal cell boundaries in *H. hockii* subsp. *colliculata* (Fig. 10F).

Literature Cited

Baker, J. G. 1876. On new bulbous plants from the eastern provinces of Cape Colony. J. Bot. 14: 181–184.

———. 1878a. Report on the Liliaceae, Iridaceae, Hypoxidaceae, and Haemodoraceae of Welwitsch's Angolan Herbarium. Trans. Linn. Soc. London, Bot. 1: 245–273.

———. 1878b. A Synopsis of Hypoxidaceae. J. Linn. Soc., Bot. 17: 93–126.

Barthlott, W. 1981. Epidermal and seed surface characters of plants: Systematic applicability and some evolutionary aspects. Nordic J. Bot. 1: 345–355.

Bentham, G. & J. D. Hooker. 1883. Genera Plantarum, Vol. 3(2). Lovell Reeve, London.

Binns, B. 1968. A First Check List of the Herbaceous Flora of Malawi. The Government Printer, Zomba.

Brackett, A. 1923. Revision of the American Species of *Hypoxis*. Contr. Gray Herb. 69: 120–147.

Britt, R. F. 1967. A Revision of the Genus *Hypoxis* in the United States and Canada. Ph.D Thesis, University of North Carolina, Chapel Hill.

Champluvier, D. 1987. Hypoxidaceae. Pp. 81–84 *in* G. Troupin (editor), Flore du Rwanda Spermatophytes, Vol. 4. Ann. Mus. Roy. Afrique Centr., Sci. Econ., Vol. 16.

De Wildeman, E. 1913. Decades novarum specierum florae katangensis. VIII–XI. Repert. Spec. Nov. Regni. Veg. 11: 537–538.

———. 1914. Notes sur la Flore du Katanga. III. Ann. Soc. Sci. Bruxelles 38(3, 4), part 2: 1–32.

———. 1921. Contribution à l'étude de la Flore du Katanga. Typo-Litho. D. Reynaert, Bruxelles.

Duvigneaud, P. 1958. La végétation du Katanga et de ses sols metallifères. Bull. Soc. Roy. Bot. Belgique 90: 127–285.

——— & S. Denaeyer-De Smet. 1963. Cuivre et végétation au Katanga. Bull. Soc. Roy. Bot. Belgique 96: 93–231.

Fries, R. 1916. Botanische Untersuchungen: Monocotyledons und Sympetale. *In:* Wissenschaftliche Ergebnisse der Schwedischen Rhodesia-Kongo-Expediton 1911–1912. Aftonbladets, Stockholm.

Geerinck, D. J. L. 1969. Genera des Haemodoraceae et des Hypoxidaceae. Bull. Jard. Bot. État. 39: 66–82.

———. 1971. Hypoxidaceae. *In:* P. Bamps (editor), Flore du Congo du Rwanda et du Burundi, Spermatophytes. Jardin botanique national de Belgique, Bruxelles.

Gillet, J. & E. Pâque. 1910. Plantes principales de la Région de Kisantu: Leur nom indigène, leur nom scientifique, leurs usages. *In:* Notes botaniques sur la région du Bas- et Moyen-Congo; fasc. I. Ann. Mus. Congo Belge, Bot., sér. 5.

Greuter, W., J. McNeill, F. R. Barrie, H. M. Burdet, V. Demoulin, T. S. Filgueiras, D. H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland & D. L. Hawksworth (editors). 2000. International Code of Botanical Nomenclature (Saint Louis Code). Regnum Veg. 138.

Heideman, E. 1983. Studies of diagnostic features in the genus *Hypoxis* L. (Hypoxidaceae R. Br.) on the Witwatersrand. Bothalia 14: 889–893.

Hilliard, O. M. & B. L. Burtt. 1978. Notes on some plants from Southern Africa, chiefly from Natal: VII. Notes Roy. Bot. Gard. Edinburgh 36: 43–76.

——— & ———. 1983. Notes on some plants of Southern Africa, chiefly from Natal: X. Notes Roy. Bot. Gard. Edinburgh 41: 299–319.

Hummel, K. & K. Staesche. 1962. Die Verbreitung der Haartypen in den natürlichen Verwandschaftsgruppen. Handbuch der Pflanzenanatomie 4(5). Gebrüder Borntraeger, Berlin.

Lebrun, J.-P. & A. L. Stork. 1991. Énumération des Plantes à Fleures d'Afrique Tropicale, Vol. 1. Conservatoire et jardin Botanique, Genève.

Linnaeus, C. 1759. Systema Naturae, ed. 10, Vol. 2. Salvii, Holmiae.

Malaisse, F. P. 1983. Phytogeography of the copper and cobalt flora of Upper Shaba (Zaïre), with emphasis on its endemism, origin and evolution mechanisms. Bothalia 14: 497–504.

Mandango, M. A. & M. Ndjele. 1994. Diversité et Centres d'Endémisme des Plantes Supérieures du Zaire. Pp. 891–901 *in* J. H. Seyani & A. C. Chikuni (editors), Proceedings of XIII[th] Plenary Meeting AETFAT, 2. National Herbarium and Botanic Gardens of Malawi, Zomba.

McVaugh, R. 1989. Liliaceae. Pp. 120–293 *in* W. R. Anderson (editor), Fl. Novo-Galiciana 15. The University of Michigan Herbarium, Ann Arbor.

Nel, G. 1914a. Studien über die Amaryllidaceae–Hypoxideae unter besonderer Berücksichtigung der afrikanischen Arten. Pp. 234–286 *in* A. Engler (editor), Beiträge zur Flora von Afrika. 43. Bot. Jahrb. Syst. 51.

———. 1914b. Die Afrikanischen Arten der Amaryllidaceae–Hypoxideae. Pp. 287–339 *in* A. Engler (editor), Beiträge zur Flora von Afrika. 43. Bot. Jahrb. Syst. 51.

Nordal, I. 1997. Hypoxidaceae. Pp. 86–87 *in* S. Edwards, S. Demissew & I. Hedberg (editors), Flora of Ethiopia and Eritrea, Vol. 6. The National Herbarium, Addis Ababa University, Addis Ababa. The Department of Systematic Botany, Uppsla University, Uppsala.

———, M. M. Laane, E. Holt & I. Staubo. 1985. Taxonomic studies of the genus *Hypoxis* in East Africa. Nordic J. Bot. 5: 15–30.

Norlindh, T. & H. V. Weimarck. 1937. Beiträge zur Kenntnis der Flora von Süd-Rhodesia. V. Bot. Not. 3–4: 161–201.

Oganezova, G. G. 1995. On the systematical position of the families Haemodoraceae, Hypoxidaceae, and Taccaceae (the data on the seed structure). Bot. Zhurn. (Moscow & Leningrad) 80: 12–25.

Richards, M. A. E. & W. V. Morony. 1969. Checklist of the Flora of Mbala (Abercorn) & District. Mbala.

Robyns, W. & R. Tournay. 1955. Monocotylées. *In:* Flore de Spermatophytes du Parc National Albert. 3. Institution des Parcs Nationaux du Congo Belge, Bruxelles.

Salisbury, R. A. 1866. The Genera of Plants. Liriogamae. Van Voorst, London.

Scharf, W. 1892. Beiträge zur Anatomie der Hypoxideen und einiger verwandten Pflanzen. Bot. Zentralbl. 52: 145–153, 177–184, 321–327.

Troupin, G. 1971. Syllabus de la Flore du Rwanda Spermatophytes. Ann. Mus. Roy. Afrique Centr., Sci. Econ., Vol. 7.

White, F. 1983. The Vegetation of Africa: A Descriptive Memoir to Accompany the UNESCO/AETFAT/UNSO Vegetation Map of Africa. UNESCO, Paris.

Williams, F. N. 1901. On *Ianthe*, a genus of Hypoxidaceae. J. Bot. 39: 289–294.

Wilsenach, R. 1967. Cytological observations on *Hypoxis*: I. Somatic chromosomes and meiosis in some *Hypoxis* species. J. S. African Bot. 33: 75–84.

——— & J. N. Papenfus. 1967. Cytological observations on *Hypoxis*: II. Pollen germination, pollen tube growth and haploid chromosome numbers in some *Hypoxis* species. J. S. African Bot. 33: 111–116.

——— & J. L. Warren. 1967. Cytological observations on *Hypoxis*: III. Embryo-sac development in *Hypoxis rooperi* and *H. filiformis*. J. S. African Bot. 33: 133–140.

Zimudzi, C. 1994. The Cytology and Reproduction of the genus *Hypoxis* L. Pp. 535–543 *in* J. H. Seyani (editor), Proceedings of XIII[th] Plenary Meeting AETFAT, 1. National Herbarium and Botanic Gardens, Malawi, Zomba.

———. 1996. A synopsis of the Hypoxidaceae in the Flora Zambesiaca area. Kirkia 16: 11–19.

List of Species

1. *Hypoxis angolensis* Baker
2. *Hypoxis angustifolia* Lam.
3. *Hypoxis bampsiana* Wiland
4. *Hypoxis canaliculata* Baker
5. *Hypoxis dinteri* Nel
6. *Hypoxis filiformis* Baker
7. *Hypoxis goetzei* Harms
8. *Hypoxis hockii* De Wild.

8a. *Hypoxis hockii* De Wild. var. *hockii*

8b. *Hypoxis hockii* var. *colliculata* Wiland

8c. *Hypoxis hockii* var. *katangensis* (Nel) Wiland

9. *Hypoxis kilimanjarica* Baker
10. *Hypoxis lejolyana* Wiland
11. *Hypoxis lusalensis* Wiland
12. *Hypoxis malaissei* Wiland
13. *Hypoxis malosana* Baker
14. *Hypoxis monanthos* Baker

15a. *Hypoxis muhilensis* Wiland subsp. *muhilensis*

15b. *Hypoxis muhilensis* subsp. *kansimbensis*

16. *Hypoxis robusta* Nel
17. *Hypoxis subspicata* Pax
18. *Hypoxis symoensiana* Wiland
19. *Hypoxis upembensis* Wiland
20. *Hypoxis urceolata* Nel

Index to Exsiccatae

* indicates type specimens

Augier 2888 (20), 2898 (2).

Bamps 851 (14), 2727 (20), 2878 (9), 3302 (9). Bamps & Malaisse 8052 (2), 8278 (2). Becquet 590 (20), 2153 (13), 2159 (14). Bequaert 3391 (2), 4211 (2), 4919* (20), 5198* (20), 5505 (2). Bouxin 429 (9), 984 (9). Bouxin & Radoux 418 (2), 434 (2), 1008 (2), 1105 (20), 1244 (20), 1415 (2). Breyne 2590 (2), 2624 (2), 4711 (2). Bulaimu 58 (5), 715 (7). Burchell *s.n.** (20). Burgeon 14 (20), 17 (2), 34 (2). Busse 947* (7).

Cabu 36 (8b). Christiaensen 736 (2), 800 (20), 1501 (9), 1553 (20), 1620 (9). Claessens 1146 (20). Cooper 462* (6). Crispiels-Thonon 132 (2).

Dandoy 206 (2), 361 (2). D'Hose 48 (5). Deschamps *s.n.** (8). Detilleux 229 (5), 383 (13). de Wilde 273 (2), 467 (2). de Witte 4 (8), 11267 (2), 1130* (2), 13341 (2), 2714 (7), 2741 (13), 2809 (7), 3698* (19), 4406 (7), 7181 (13), 7307 (7), 7364 (7), 7451 (7), 7508 (2). Dinter 634* (5). Doumen 9 (13). Dupuis *s.n.* (2). Duvigneaud 1311 (13), 1319 (13), 1321H (1), 1324 (13), 4129 (13), 4130 (17), 4130 H_1 (13), 4312 (13), 4471(13), 4527 H (1), 4532H (10), 4861 (13), *s.n.* (1), *s.n.* (16). Duvigneaud & Timperman 2175 (16), 2209 (16), 2213 (16), 2264 (1), 2400 (7), 2404 (13), 2406 (13), 2481 (1), 2514 (1), 2519 (10), 2528 (17), 2551 (16), 2626 (13), 2627 (7), 2697 (13), 2700 (7), 2731 (7).

Ecklon 3515* (6).

Federicq in de Witte 8741 (2). Flamigni 297 (2). Foscolos 19 (20). Fries 1148 (8c), 1275 (4). Froment 681 (9).

Germain 2854 (20), 3962 (20), 6086 (2), 6237 (2), 7191 (2). Gillet *s.n.* (2), *s.n.* (2). Goetze 1416* (7).

Heine 108 (13), U 111 (13). Hendrickx 4780 (9), 4832

(9). Hens 221 (2). Hildebrandt 2542* (20). Hock *s.n.** (7), *s.n.* (7), *s.n.** (8a), *s.n.* (8a), *s.n.** (13), *s.n.* (13). Homblé 809 (13), 1091 (2). Hooper & Townsend 532 (19).

Jans 98 (2). Johnston 1117 (20).

Kinet 49 [= Hendrickx 4361] (13). Kisimba, Muzinga, Matamba 136 (13).

Lebrun 3256 (2), 3755 (20), 7557 (2), 7835 (2), 9529 (20). Lejoly 2949A (20), 2956 (20), 3338 (2), 82/1013 (2), 84/719 (9). Lewalle 584 (9), 1102 (9), 3997 (9), 4189 (2), 5029 (2), 5130 (9), 5486 (2). Liben 620 (2), 657 (20), 1231 (2). Liebusch *s.n.** (9). Liney 212 (5). Lisowski 7274 (13), 7655 (7), 8023 (13), 10710c (14), 48204 (20), 48484 (2), 50994 (7), 56805 (2), 81107 (7), 81109 (13), 81116* (15a), 81120 (14), 81121 (13), 81122 (13), 81123 (4), 81124 (13), 81125* (15b), 81134 (15a), 81145 (13), 81146 (13), 82460 (13), 82893 (13), 86119 (2), 92473 (13), B-7173A (14), B-7173B (13), B-7231 (13), B-7270 (7), B-7270A (13), B-7271 (13), B-7272 (13), B-7273 (13), B-7274 (13), B-7276 (2), B-7277 (13), B-7278 (22), B-7279A (13), B-7378 (2). Lisowski, Malaisse & Symoens 178 (10), 184 (14), 737 (3), 810 (13), 1009 (3), 1155 (13), 2652 (14), 5637 (1), 7413 (8), 7470 (13), 7477 (13), 7492 (7), 7565 (8a), 7596 (13), 7615 (3), 7653* (3), 7654 (8b), 7682* (8b), 7744 (13), 7822 (13), 8125 (13), 8134A (13), 8268 (14), 8300 (11), 8369 (13), 8453* (11), 8557 (11), 8574a (8b), 8645 (8), 8665 (7), 8669 (7), 8684 (11), 9557 l (2), 10710A (2), 11797 (8c), 11798 (7), 12058* (18), 12058A (13), 12573 (14), 12830 (3), 12853 (3), 12859 (3), 12947 (3), 12984 (16), 13279 (1), 13531 (13), B-7279 (3). Louis 4587 (2). Lukuesa 116 (1).

Malaisse 6042 (18), 6100 (13), 7403* (12), 8988 (2), 13562 (13), 13695 (8b), 13696 (7). Menager 67 (20). Michel 2534 (14), 5007 (9). Mortelmans 89 (7). Mullenders 2346 (2).

Nicolas 502 (1). Niyongere 95 (6). Noirfalise 826 (2).

Obst *s.n.** (20). Odon in Gillet *s.n.* (2). Overlaet 712 (10).

Pauwels 6009 (2), 6922 (8b). Plancke 63/911 (7), 127–1741 (7), 158/2184* (10), 159/2206 (13), 159/2214 (13), 167/2550 (1), 167/2613 (18). Pogge 424 (17).

Quarré 704 (7), 719 (13), 765 (8b), 921 (7), 2673 (8a), 3416 (7), 3527 (13), 3717 (13), 4314 (13), 5056 (2), 6035 (7), 7252 (7), 7252 (11), 7256 (13).

Rameloo 4775 (13). Reekmans 23 (9), 756 (9), 1335 (9), 2065 (9), 2906 (2), 5719 (14). Richards 27606 (17). Risopoulos 776 (17). Robyns 3553 (20), 3587 (7), 3604 (13), 3891 (16).

Schaijes 2398 (13), 2985 (1), 3032 (13). Schmitz 2109 (13), 2977 (1), 4198 (13), 4199 (8b), 4803 (7), 5855 (13), 5921 (16), 7499 (7), 7675 (13). Scops 168 (2). Smets 13 (9). Sperry 353 (20). Spyut & Muchali 3462 (1). Staner 2077 (13). Stauffer 500 (2). Streel 331(13). Symoens 779 (13), 5886 (1), 13672 (13).

Taton 437 (2), 957 (20). Teuscz in von Mechow 249* (17), Troupin 1243 (2), 1308 (2), 2699 (20), 4230 (2), 4800 (20), 5287 (20), 6776 (2), 8199 (20), 8606 (20), 8864 (20), 9474 (20), 13969 (20), 14108 (20), 14109 (2).

Van der Veken 10754 (20). Van Oosten 106 (20), 120 (2). Vanderyst 37242 (2), *s.n.* (2), *s.n.* (2). Verdick 198 (16), *s.n.* (16), *s.n.** (8c). Volkens 360* (20), 781* (9).

Wagemans 285 (2). Welwitsch 4057* (14), 4058* (4), 4059* (1). Whyte *s.n.** (13). Wilbaux 167 (20). Williams 18 (2).

THE ESTABLISHMENT OF THE NEW GENUS *ALTOPARADISIUM* AND A REEVALUATION OF *ARTHROPOGON* (POACEAE, PANICEAE)[1]

Tarciso S. Filgueiras,[2] *Gerrit Davidse,*[3] *Fernando O. Zuloaga,*[4] *and Osvaldo Morrone*[4]

ABSTRACT

Altoparadisium is described as a new genus of Paniceae based on *A. chapadense*, sp. nov., from the campo rupestre habitats of Goiás, Brazil. *Altoparadisium chapadense* is illustrated and fully described morphologically and anatomically. It is extremely unusual in the Paniceae in possessing only two spikelet bracts and in having spikelet reduction from the apex as well as the base. The bracts are interpreted to be homologous to an upper glume and lower lemma. The only flower is interpreted to be homologous to the upper flower of a typical panicoid spikelet. It is a C_4 grass with Kranz anatomy, distinctive Kranz cells, and typical panicoid bicellular microhairs. In order to establish the phylogenetic relationships of *Altoparadisium*, it was included in a cladistic analysis of *Arthropogon* and related genera. We conclude that: (1) *Arthropogon* as traditionally circumscribed is polyphyletic; (2) *Arthropogon scaber* is the sister species of *Altoparadisium chapadense*; it is therefore transferred to this genus as *Altoparadisium scabrum* and illustrated for the first time; (3) *Arthropogon bolivianus* and *A. rupestris* are reduced to varieties of *Altoparadisium chapadense*; (4) *Arthropogon piptostachyus* is recognized as originally described, i.e., as the monotypic genus *Achlaena*; (5) *Arthropogon lanceolatus* should probably be included in a new, monotypic genus, but because its phylogenetic relationships remain unresolved, such a new name is not yet proposed. The recircumscribed genera *Arthropogon* and *Achlaena* are briefly summarized, and a key to their species and varieties is provided.

Key words: *Achlaena*, *Altoparadisium*, anatomy, *Arthropogon*, Arthropogoninae, Brazil, Paniceae, Poaceae, phylogeny, taxonomy.

Chapada dos Veadeiros is one of the 25 physiographic units reported for Brazil by Cochrane et al. (1985). It is located between 13–15°S and 47–49°W, and the altitude varies from 800 to 1700 m. The vegetation is made up principally of campo rupestre but also gallery forests, cerrado (sensu stricto), campo limpo of cerrado, and permanent marshes. The flora of Chapada dos Veadeiros is poorly known. A botanical survey undertaken by Munhoz and Proença (1998) found 106 families, 372 genera, and 751 species, 66 of which were considered endemic. One notable recent discovery from the Chapada dos Veadeiros is a new species of *Triraphis* R. Br. (Poaceae), *T. devia* Filg. & Zuloaga, which documents a New World occurrence for a genus previously thought to be disjunct between Africa and Australia (Filgueiras & Zuloaga, 1999).

During a field excursion to the Chapada dos Veadeiros in September 1994 as part of the project "Biogeography of the Cerrado Biome" (Felfili et al., 1994, 1997), a morphologically unusual grass was collected that seemed to be an undescribed species and could not immediately be assigned to any described genus in the Poaceae (Clayton & Renvoize, 1986; Nicora & Rúgolo de Agrasar, 1987; Webster et al., 1989; Watson & Dallwitz, 1992).

As detailed below, with further studies we indeed determined that this grass represented an undescribed genus, and it is here described and named as *Altoparadisium chapadense*. In order to finalize this conclusion, we also had to consider the relationship of *Altoparadisium* with genera traditionally

[1] We thank J. E. de Paula (UB) for his help in the preparation of the anatomical slides and photographs, Neil Snow for SEM photographs, H. Ziegler for the carbon isotopic analysis, Vladimiro Dudás, San Isidro, Argentina, for the excellent illustrations in Figures 1 and 7, and Victoria C. Hollowell and two anonymous reviewers for many suggestions that improved the final version of the paper. The senior author thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a fellowship (process #301190/86-0). The fieldwork for this project was funded by the Biogeografia do Bioma Cerrado project and by a grant from the National Geographic Society.

[2] UPIS-Faculdades Integradas, Dept. de Agronomia, Campus II, Cx. Postal 10743, Planatina, DF 70330-980, Brazil.

[3] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A.

[4] Instituto de Botánica Darwinion, Labardén 200, Casilla de Correo 22, San Isidro 1642, Argentina.

included in subtribe Arthropogoninae (Clayton & Renvoize, 1986: 300). This additionally led us to test the monophyly of *Arthropogon*, to investigate the sister-group relationships of *Arthropogon*, and to revise the taxonomy of the species traditionally included in *Arthropogon*.

Materials and Methods

The morphological and anatomical studies were based on herbarium specimens from IBGE, MO, SI, and US and supplemented with observation from living specimens cultivated at IBGE. In the taxonomic treatment, we only cite additional voucher specimens not already studied and listed previously by Filgueiras (1982, 1986, 1996).

The standardized terminology for comparative leaf anatomy proposed by Ellis (1976, 1979) was used. Cross-sectional leaf anatomy was determined from hand-sectioned cross sections of leaf blades of herbarium specimens previously treated with ethylene glycol for 24–48 hours. The blades were sectioned approximately one-third from the base, mounted on permanent slides, and then stained using standard anatomical techniques. Some sections were studied without clearing, in order to determine the relative position of the chloroplasts in the Kranz sheaths. The rest were cleared and stained with Alsatian Blue-safranin (Cutler, 1978) or safranin-fast green.

Epidermal preparations for light microscope studies were made following the methodology of Metcalfe (1960). The SEM epidermal studies were done using a Zeiss 940A scanning electron microscope at the Instituto de Botánica Darwinion, Buenos Aires, Argentina.

For the cladistic analysis we used 28 morphological characters and 6 anatomical characters (Appendix 1, Table 1). Two of these (14, 26) are multistate characters and were treated as non-additive (unordered). Most of the data (90%) were obtained directly from herbarium specimens and supplemented with published data. Table 2 contains the data matrix, which was analyzed using Hennig86 version 1.5 (Farris, 1988), applying the option of implicit enumeration, i.e., all polymorphic entries were replaced in the data matrix (Table 2) as missing entries. In parallel, the data matrix was analyzed under parsimony with equal weighting using Nona, version 1.8 (Goloboff, 1993). This analysis was conducted using the default setting *amb-* (clades resolved only if they have unambiguous support) and *poly* = (polytomies allowed), and also utilizing the command mswap*13. Consistency indexes (CI, Kluge & Farris, 1969), and retention indexes (RI, Farris, 1989), were calculated only for informative characters. Bremer support, the number of extra steps needed to lose a branch in the strict consensus of near-most-parsimonious trees (Bremer, 1994), was calculated in order to evaluate the relative support of clades. Similarly, bootstrap analysis was also performed (Felsenstein, 1985). The calculation of branch support values and bootstrap analysis was done with Nona (Goloboff, 1993).

For the construction of the consensus tree, we used the Nelson option of the cited programs. CLADOS (Nixon, 1993) was used for the analysis of the

Table 1. List of the characters and character states used in the cladistic analysis.

1. Inflorescence: open (0) closed (1)
2. Pilose callus: absent (0) present (1)
3. Spikelet compression: dorsal (0) lateral (1)
4. Internode between glume 1 and glume 2: absent (0) present (1)
5. Lower glume: absent (0) present (1)
6. Upper glume apex: muticous (0) mucronate or awned (1)
7. Upper glume apex: entire (0) bilobed (1)
8. Lower lemma apex: muticous (0) awned (1)
9. Lower lemma apex: entire (0) bilobed (1)
10. Lower palea: absent (0) present (1)
11. Lower flower: absent (0) present (1)
12. Upper floret compression: dorsal (0) lateral (1)
13. Upper lemma: absent (0) present (1)
14. Upper palea, relative length in relation to the upper lemma: 1/1 (0) 1/4–3/4 (1) 1/4 (2) absent (3)
15. Upper lemma texture: cartilaginous (0) hyaline (1)
16. Lodicules of the upper floret: absent (0) present (1)
17. Functional stamens: three (0) two (1)
18. Parenchyma sheath: absent (0) present (1)
19. Mestome sheath: non-Kranz (0) Kranz (1)
20. Distinctive Kranz cells: absent (0) present (1)
21. Fusoid cells: absent (0) present (1)
22. Intercostal grooves of the upper glume: absent (0) present (1)
23. Vascular bundles below the bulliform cells: absent (0) present (1)
24. Leaf blades: flat (0) cylindrical (1)
25. Hilum type: punctate (0) linear (1)
26. Upper floret color: straw-colored (0) castaneous (1) translucent (2)
27. Germination lid: absent (0) present (1)
28. Upper glume texture: coriaceous (0) herbaceous (1)
29. Lower lemma texture: coriaceous (0) herbaceous (1)
30. Vascular bundles: ordered (0) unordered (1)
31. Awn of the upper glume: straight (0) twisted (1)
32. Spikelet hairs: eglandular (0) glandular (1)
33. Spikelet base: not toroid (0) toroid (1)
34. Lower glume: without rigid tuberculate hairs (0) with tuberculate hairs (1)

Table 2. Data matrix used in the cladistic analysis of Altoparadisium and related genera. Square brackets "[]" indicate polymorphism in binary characters. For a taxon a hyphen (-) indicates character inapplicable and a question mark (?) indicates character unobserved.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Thyridolepis	1	1	0	0	1	0	0	0	0	[0 1]	[0 1]	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	-	0	0	1
Homolepis	0	0	0	0	1	0	0	0	0	1	[0 1]	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	1	1	0	-	1	0	0
Streptostachys	0	0	0	0	1	0	0	0	0	[0 1]	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	1	1	0	-	0	1	0
Digitaria	0	0	0	0	1	0	0	0	0	[0 1]	[0 1]	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	0	-	0	0	0
Homopholis	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	-	0	0	0
Melinis	0	0	1	0	1	1	1	1	1	[0 1]	[0 1]	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Leptocoryphium	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	1	1	1	-	0	0	0
Anthaenantia	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	1	1	1	1	0	-	0	0	0
Reynaudia	1	0	1	0	1	1	1	1	1	0	0	1	1	3	1	0	1	0	1	0	0	0	0	0	1	2	0	1	1	0	0	0	0	0
Achlaena	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Altoparadisium chapadense	1	1	0	0	0	1	0	0	0	0	0	0	0	3	-	1	0	0	1	1	0	1	0	0	0	-	-	1	1	0	1	0	0	0
Altoparadisium scabrum	1	1	0	0	1	1	0	0	0	[0 1]	0	0	1	2	1	1	0	0	1	1	0	1	0	0	0	2	0	1	1	0	0	0	0	0
Arthropogon villosus	0	1	1	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0
Arthropogon filifolius	0	1	1	0	1	1	0	0	0	1	1	1	1	1	1	1	0	0	1	0	0	0	-	1	?	2	0	0	0	0	0	0	0	0
Arthropogon xerachne	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1	0	?	2	0	0	0	0	0	0	0	0
Arthropogon lanceolatus	1	0	0	0	1	1	0	0	0	1	1	0	1	0	0	1	0	1	0	0	1	0	0	0	?	0	1	1	1	0	0	0	0	0

distribution of the characters in the cladograms and the generation of the different trees.

The taxa included in this analysis are listed in Appendix 2. For the outgroup selection we followed the work of Zuloaga et al. (2000), who analyzed the tribe Paniceae on the basis of morphological and anatomical characters. In this study, Zuloaga et al. (2000) treated *Arthropogon* as three monophyletic subunits. The results indicated that these three subunits were each related to different and distinct taxa of Paniceae, although this analysis could not completely resolve all generic relationships. Consequently, the following taxa were selected as outgroups for *Arthropogon* sensu lato: *Anthaenantia* P. Beauv., *Digitaria* Haller, *Homolepis* Chase, *Leptocoryphium* Nees, *Melinis* P. Beauv., *Reynaudia* Kunth, *Streptostachys* Desv., *Homopholis* C. E. Hubb., and *Thyridolepis* S. T. Blake. Among these genera, the tree was rooted in *Thyridolepis* on the basis of possessing the C_3 photosynthetic pathway, which has been suggested to be ancestral to the C_4 pathway (Brown, 1977; Kellogg & Campbell, 1987). Also, Clayton and Renvoize (1986: 267) placed the subtribe Neurachninae (S. T. Blake) Clayton & Renvoize (= Neurachneae S. T. Blake, Watson & Dallwitz, 1992) at the base of the tribe Paniceae and proposed that the inclusion of taxa with non-Kranz anatomy, with the upper lemma not specialized, and the presence of 3 stigmas suggests an early split from the mainstream Paniceae. Similarly, Kellogg and Watson (1993) rooted the trees for their study of the Andropogodae L. Liou at *Thyridolepis*; and recently Wills et al. (2000), in their revision of *Homopholis*, also rooted the trees in the Neurachneae and *Thyridolepis*.

Spikelet Morphology and Leaf Anatomy of *Altoparadisium chapadense*

The subfamilial and tribal affinities of the majority of grass species can usually be very readily deduced from spikelet and inflorescence morphology alone. However, that was not the case with *Altoparadisium chapadense*. Its spikelets consist of only two bracts (Fig. 1C–F). On the one hand, its very simple spikelet makes it distinctive, but on the other hand, this very simplicity makes an interpretation of the homology of the spikelet bracts difficult. For this reason we relied primarily on anatomical evidence to deduce its tribal affinities. From this basic anatomical deduction, we then considered a number of hypotheses of spikelet interpretation based on trends in spikelet morphology of putative relatives, especially *Arthropogon*.

Altoparadisium chapadense has a C_4 anatomical organization. This is most clearly evidenced from the presence of only 2 or 3 chlorenchyma cells between all adjacent vascular bundles, a character that is most highly correlated with grass species that have C_4 photosynthesis (Hattersley & Watson, 1975). In addition, since no cells intervene between the metaxylem vessel elements and the laterally adjacent chlorenchymatous, bundle sheath (Kranz) cells in the primary lateral vascular(XyMS-), this indicates that it probably is an NADP-ME type genus (Hattersley & Watson, 1975, 1976).

Additional confirmation of the C_4 status of *A. chapadense* was provided through an analysis of ^{12}C and ^{13}C ratios. It has been firmly established that $\delta^{13}C$ values of -9 to -16% indicate C_4 photosynthesis, while $\delta^{13}C$ values of -23 to -32% indicate C_3 photosynthesis (Smith & Brown, 1973). A leaf sample of a potted plant of *A. chapadense* was analyzed by H. Ziegler (Institut für Botanik und Mikrobiologie, Technische Universität, München, Germany) for its $\delta^{13}C$ value, with the following result: -11.82 (Ziegler, pers. comm.). Thus, according to well-established criteria, the value obtained in this analysis is an independent confirmation that *A. chapadense* is a C_4 species.

The cross-sectional leaf anatomy of *Altoparadisium* is especially distinctive due to the occurrence of isolated bundle sheath cells, known as distinctive Kranz cells (Sánchez & Arriaga, 1989; Sánchez et al., 1989), in the mesophyll between adjacent vascular bundles (Fig. 2B). These cells have also been called distinctive cells (Tateoka, 1958), circular cells (Metcalfe, 1960), axillary bundle cells (Renvoize, 1982a, b), and specialized parenchyma cells (Reger & Yates, 1979). As noted by Renvoize (1982a), these distinctive Kranz cells are similar in structure and function to bundle sheath cells, and are the equivalent to a vascular bundle and bundle sheath reduced to a single cell (Tateoka, 1958). The number of chlorenchymatous mesophyll cells between adjacent vascular bundles and adjacent distinctive Kranz cells or between adjacent distinctive Kranz cells is two or three. This is a strong indication that *Altoparadisium* is a C_4 genus (Hattersley & Watson, 1975). Distinctive Kranz cells have so far only been reported in subfamily Panicoideae in five genera of the tribe Arundinelleae and four genera of Paniceae (reviewed in Morrone et al., 1995, who list one additional genus, *Achlaena*, a genus included in *Arthropogon* by Filgueiras, 1982). This is good evidence that *Altoparadisium* belongs in the Panicoideae.

Finally, the occurrence of microhairs of the two-celled, thin-walled type with a tapering distal cell, usually called the panicoid type, is additional,

Figure 1. *Altoparadisium chapadense* (based on *Filgueiras & Fonseca 2987*). —A. Habit. —B. One spikelet pair and two solitary spikelets on the ultimate portion of an inflorescence branch; note prominent awn of the upper glumes (awn of lowest spikelet not drawn). —C. Attached spikelet seen from the lower lemma side with prominent hairs at the apex of the pedicel and the base of the callus. —D. Lower glume, 3-nerved. —E. Detached spikelet seen from the lower lemma side with prominent callus hairs and laterally exserted stigmas. —F. Lower lemma, ventral view, with 2 lodicules at the base. —G. Caryopsis, embryo side. —H. Caryopsis, hilum side. —I. Ligule. All drawings by Vladimiro Dudás.

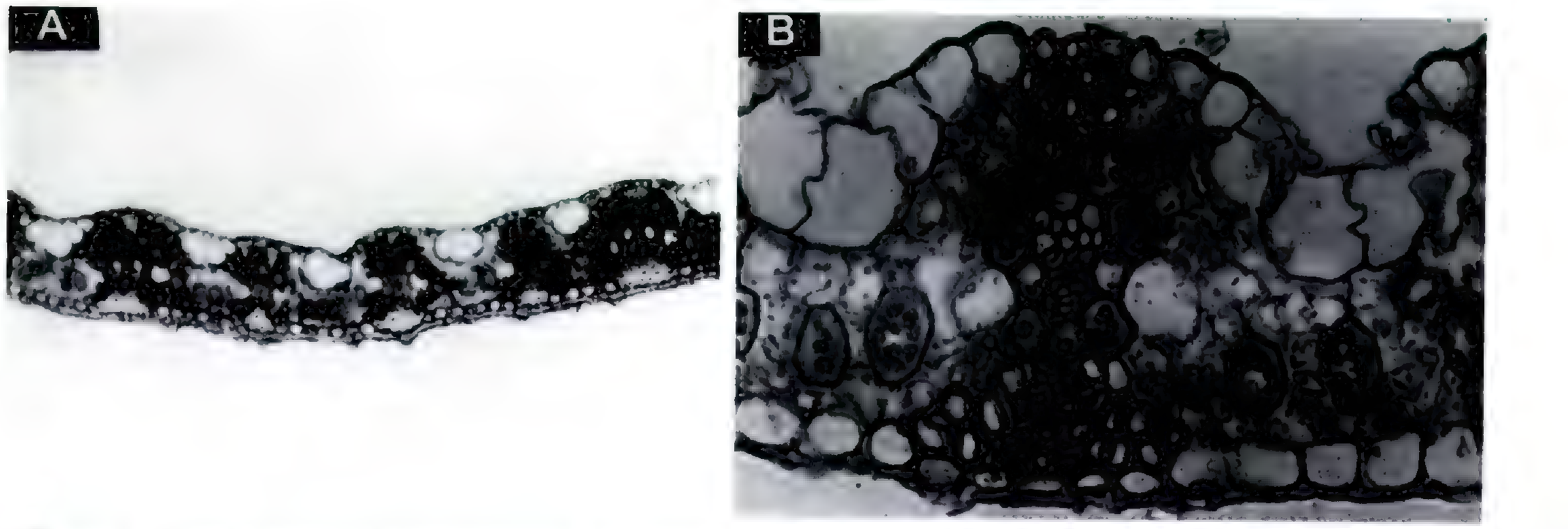


Figure 2. Leaf anatomy of *Altoparadisium chapadense*, cross-sectional leaf anatomy (based on *Filgueiras & Fonseca 2987*). —A. Cross section of a portion of the leaf blade, showing the midrib with a single first-order vascular bundle, three third-order vascular bundles, and one first-order lateral bundle. —B. Cross section of a third-order vascular bundle; note the prominent outer parenchyma sheath and the three distinctive Kranz cells on each side of the bundle.

strong evidence that *Altoparadisium* belongs in the Panicoideae (Fig. 3C, D) (Watson & Dallwitz, 1992).

Only one previously described panicoid grass has spikelets consisting of only two bracts and a single flower, *Paspalum soukupii* Carbonó, a species of the *Racemosa* group (Morrone et al., 1995). However, in *P. soukupii* the inflorescence consists of unilateral, winged racemes, totally different from the verticillate panicle of *Altoparadisium*. Furthermore, the spikelets of *Paspalum soukupii* unambiguously represent an upper floret with the typical palea and lemma of species of the group *Racemosa* or, indeed, any species of *Paspalum*. And, although distinctive Kranz cells do occur in *P. soukupii*, as well as all other nine species of *Paspalum* group *Racemosa* (Morrone et al., 1995: 112), spikelet and inflorescence morphology conclusively eliminate this group of species from further consideration as a possible close relative.

From these considerations we concluded that *Altoparadisium* represented an undescribed genus, and we continued our search for putatively related genera among the other eight genera with distinctive Kranz cells (Morrone et al., 1995). Spikelet structure is quite different in all genera of Arundinelleae, and we did not consider this tribe further. Similarly, *Anthephora* Schreb., *Dissochondrus* (Hillebr.) Kuntze, and *Paspalum* L., three of the genera of Paniceae R. Br. with distinctive Kranz cells also seem quite different in specialized spikelet and inflorescence morphology and are, therefore, probably not good candidates as possible relatives. However, in inflorescence architecture, spikelet shape and size, ligule type (a minute ring of hairs), and the ring of callus hairs at the base of the spikelet, *Altoparadisium* resembles some species of *Arthropogon*. Specifically, the inflorescence of *Altoparadisium* resembles that of *Arthropogon scaber*, *A. bolivianus*, and *A. rupestris* (Filgueiras, 1982, 1986, 1996) in being a contracted terminal panicle with a tough rachis, pilose nodes, and verticillate branches, but it differs in being pointed at the apex, and in its whitish not greenish or stramineous color. *Altoparadisium* further differs from *Arthropogon* species by the presence of a ring of hairs at the apex of the pedicel, its soft spikelet texture (membranous, not coriaceous), and the possession of only two spikelet bracts, instead of four, five, or six.

Because of the resemblance in the anatomy and inflorescence structure, and because both genera occur in the same geographical region, we hypothesized that *Arthropogon* may be the closest extant relative of *Altoparadisium*. The interpretation of spikelet structure in *Altoparadisium* was, therefore, primarily made with reference to variation patterns in *Arthropogon* and secondarily to the closely related *Reynaudia* Kunth, a monotypic Cuban genus. For the purpose of this discussion, we follow the classification of Clayton and Renvoize (1986), who place *Arthropogon* and *Reynaudia* in the subtribe Arthropogoninae Butzin of the tribe Paniceae. The recognition of the subtribe is based primarily on the more strongly indurate texture of the glumes and lower lemma compared to that of the upper floret, as well as the laterally compressed and solitary spikelets. *Arthropogon* and *Reynaudia* spikelets typically have two glumes and two florets. Although the spikelets are usually said to be laterally compressed (Filgueiras, 1982, 1986; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992), *Arthropogon* as traditionally circumscribed is actually heterogeneous in this regard (Zuloaga et al., 2000),

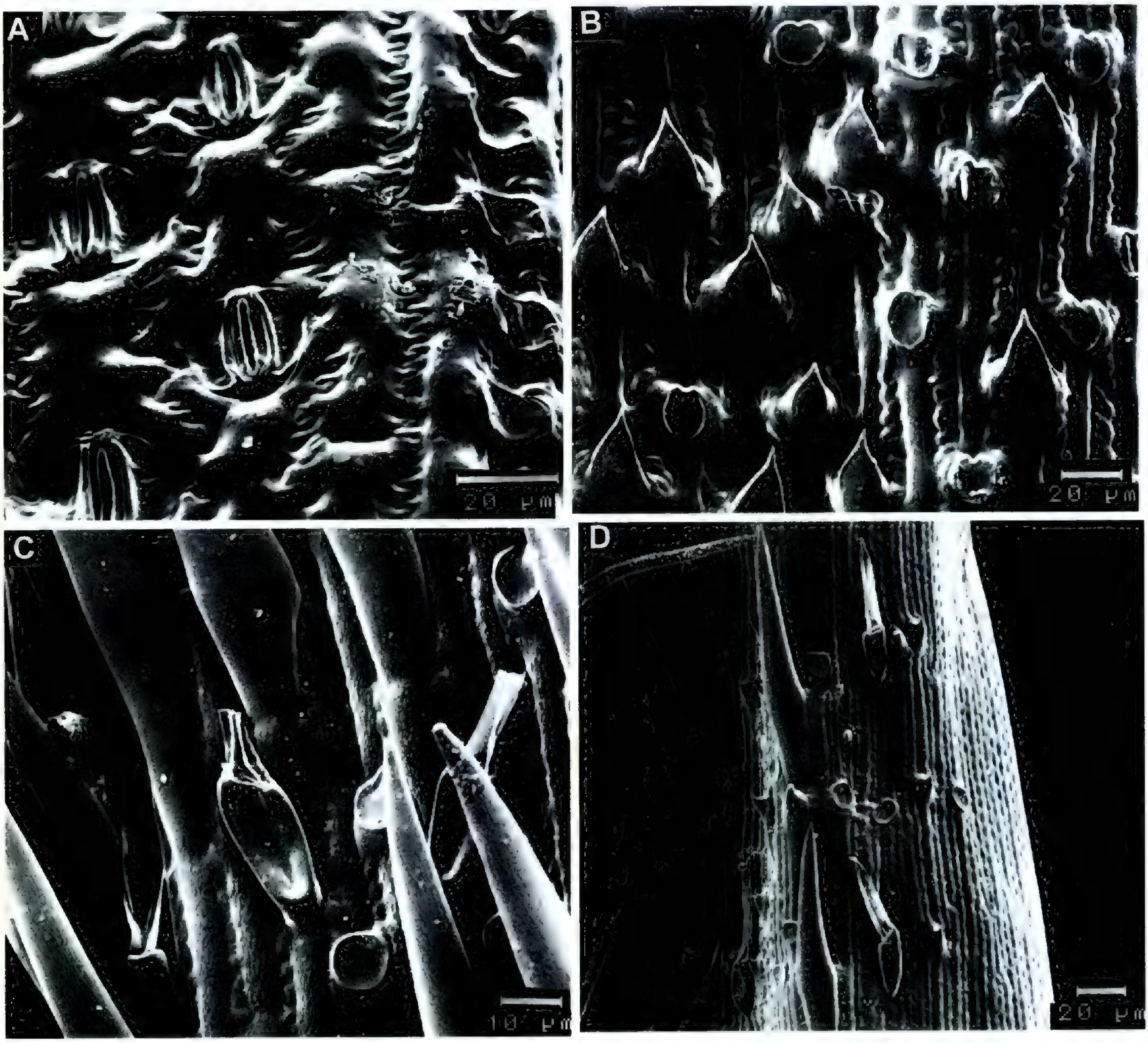


Figure 3. SEM photographs of leaf, palea, and lemma epidermal anatomy of *Altoparadisium chapadense*, in surface view (based on *Filgueiras & Fonseca 2987*). —A. Abaxial surface of the leaf blade with stomata and long cells in the intercostal zone, and silica bodies and long cells in the costal zone. —B. Adaxial surface of the leaf blade with silica bodies, prickles, and long cells. —C. Detail of the apex of the upper glume with macrohairs, bicellular panicoid microhairs, prickles, and a few silica bodies. —D. Detail of the apex of the lower lemma with numerous macrohairs, bicellular panicoid microhairs with the apical cells collapsed, and silica bodies.

as well as in several other important diagnostic characters, such as presence of a pilose callus, texture of the upper floret, hilum type, and anatomical characters. In *A. scaber, A. rupestris,* and *A. bolivianus* the spikelets are clearly dorsally compressed.

The following patterns of variation in spikelet parts are known in *Arthropogon* and *Reynaudia.* Bract number in *Arthropogon* species is six, five, or four and in *Reynaudia* four. In both genera the glumes are coriaceous or firmly membranous and awned. The lower glume varies from an awned, broad-based membrane to just an awn; the upper glume always has a fully developed membrane and is awned. The lower floret is always present. The lower flower is staminate or absent. The lower lemma generally has the same texture as the upper glume but bears no or only a small awn. The lower palea varies from 4/5 as long as the lemma to absent, and when present, is hyaline. The upper floret is always present and always has a bisexual flower. The upper lemma is always present but is reduced in size compared to the lower lemma. The upper lemma is membranous or, more commonly, hyaline in texture and may totally lack nerves. The upper palea varies from as long as the upper lemma, to half as long or entirely absent. When present it is hyaline, and when well developed, it has a typical 2-keeled, 2-nerved morphology as present in most grass genera.

We used the following criteria in our interpre-

tation of *Altoparadisium* spikelet morphology and bract homology.

(1) Since neither of the bracts is 2-keeled, it is unlikely that either represents a palea, although there are a significant number of exceptions to two-keeled paleas in the family. For example, in the DELTA Grass Genera of the World database, Watson and Dallwitz (1992 onward) list 63 genera as having at least some species with nerveless paleas, 23 genera with 1-nerved paleas, 71 genera with multinerved paleas, and 579 genera with 2-nerved paleas.

(2) Since both lower and upper paleas are variably developed in *Arthropogon* and entirely absent in *Reynaudia*, this suggests the possibility that they may have been lost entirely in *Altoparadisium*.

(3) Since the lower flower is quite variable in its development in the Panicoideae, including *Arthropogon* species, where they vary from male flowers to completely absent, and totally absent in *Reynaudia*, we hypothesize that the lower flower is also entirely absent in *Altoparadisium*. This then means that the solitary flower of *Altoparadisium* is homologous to the upper flower typically present in panicoids.

(4) Since the lower glume of *Arthropogon* is variably developed, this may indicate that it disappeared in *Altoparadisium*.

(5) Since the upper lemma is more variable in its development among *Arthropogon* species than the lower lemma, we hypothesize that it has also disappeared in *Altoparadisium*.

(6) In this process of elimination, the remaining bracts would, therefore, be homologous to the upper glume and the lower lemma of the typical panicoid spikelet. As in *Arthropogon* species and in *Reynaudia*, the texture of the upper glume is the same as that of the lower lemma. Oddly enough, these two bracts subtend the only remains of the upper floret, the upper flower.

Early in our study of *Altoparadisium* we considered a number of other interpretations of the homology of the spikelet bracts including the following: the two bracts represent (1) an upper lemma and upper palea, (2) a lower and upper glume, (3) a lower and upper lemma. In the absence of developmental studies, which might provide more definite evidence but which were beyond the scope of this project, the decisive evidence for the interpretation presented above was a side-by-side comparison of the spikelets of *Altoparadisium chapadense* and *Arthropogon scaber*. The overall gestalt of the two spikelets is very similar. If one then imagines the absence of the lower glume and upper lemma (Fig. 7), then in the texture, compression, and confirmation of the nerves and internerves, the remaining two bracts of such an *A. scaber* spikelet look very much like the corresponding bracts of the *Altoparadisium* spikelet (Fig. 1).

Summarizing, in Arthropogoninae (sensu Clayton & Renvoize, 1986) we hypothesize a reduction series in spikelet morphology that proceeds primarily from the apex to the base and secondarily from the base to the apex. In contrast to the sequence just postulated, the most common pattern of spikelet reduction in the rest of the Paniceae usually proceeds from the base to the apex and results in a tendency to reduce the lower glume before the upper glume and the lower floret and its component parts before the upper floret and its component parts (see Cialdella & Vega, 1996, and references cited therein).

Once this initial anatomical and morphological analysis had been completed, it then became evident that in order to gain a better understanding of the relationships of *Altoparadisium chapadense*, it would be desirable to do a phylogenetic analysis of any of the putatively related taxa.

Discussion

The cladistic analysis of the data matrix (Table 2) gave three equally parsimonious cladograms, using Hennig86; each cladogram was 57 steps long, with a consistency index of 0.52, and a retention index of 0.72 (Fig. 4). One of these cladograms (Fig. 6) is illustrated, including all character state changes. The strict consensus tree is shown in Figure 5, along with the Bremer support values and bootstrap percentages for each node.

A single equally parsimonious cladogram was obtained from the data matrix using Nona; this cladogram has an identical topology to the one illustrated in the strict consensus tree of Figure 5.

Monophyly of *Arthropogon*

In order to test the monophyly of *Arthropogon* and search for a sister to *Altoparadisium*, we included the genera *Melinis* (subtribe Melinidinae), *Reynaudia* (Arthropogoninae), *Homopholis*, *Digitaria*, *Leptocoryphium*, and *Anthaenantia* (Digitarinae), and *Homolepis* and *Streptostachys* (Setariinae). All taxa were rooted with *Thyridolepis* (Neurachninae).

The analysis showed that *Arthropogon* as traditionally circumscribed is polyphyletic (Fig. 6 shows one of the three trees with all characters). *Arthropogon lanceolatus* is the most basal member of the ingroup and could be circumscribed as a monotypic genus; it forms a monophyletic clade with *Streptostachys* + *Homolepis*, which is supported by the presence of fusoid cells (character 21). The boot-

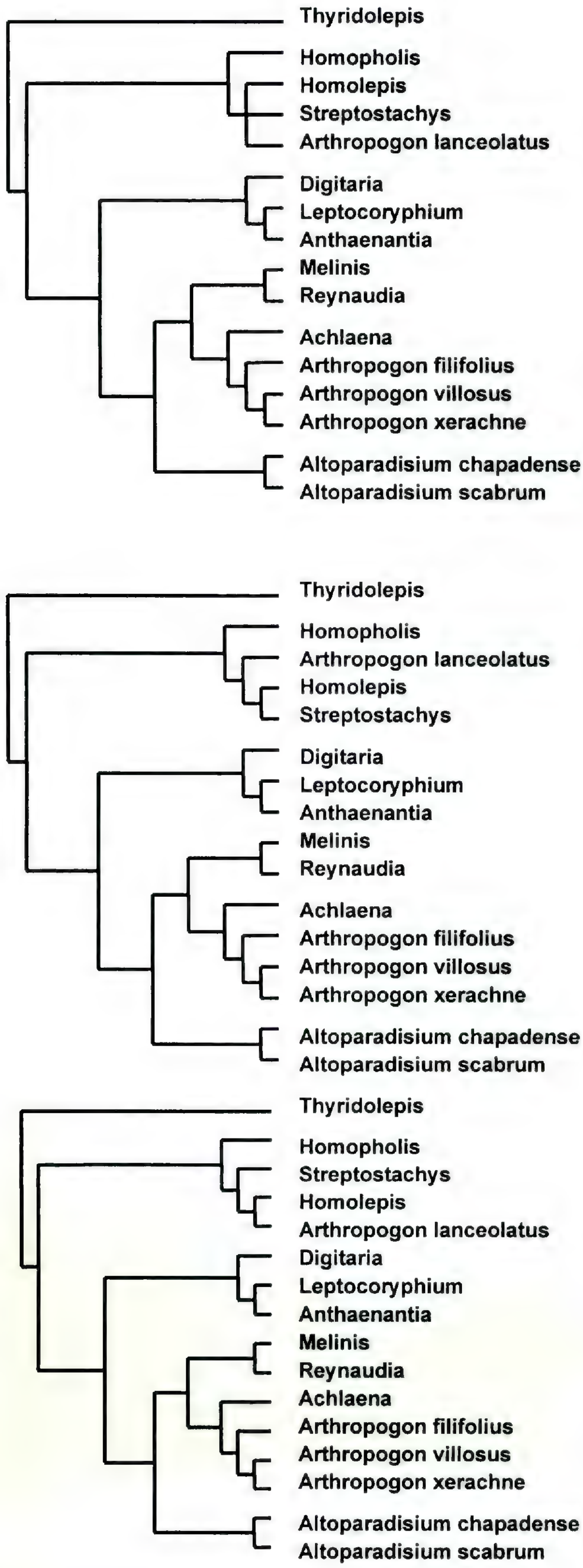


Figure 4. Equally parsimonious trees obtained from the data matrix (Table 2).

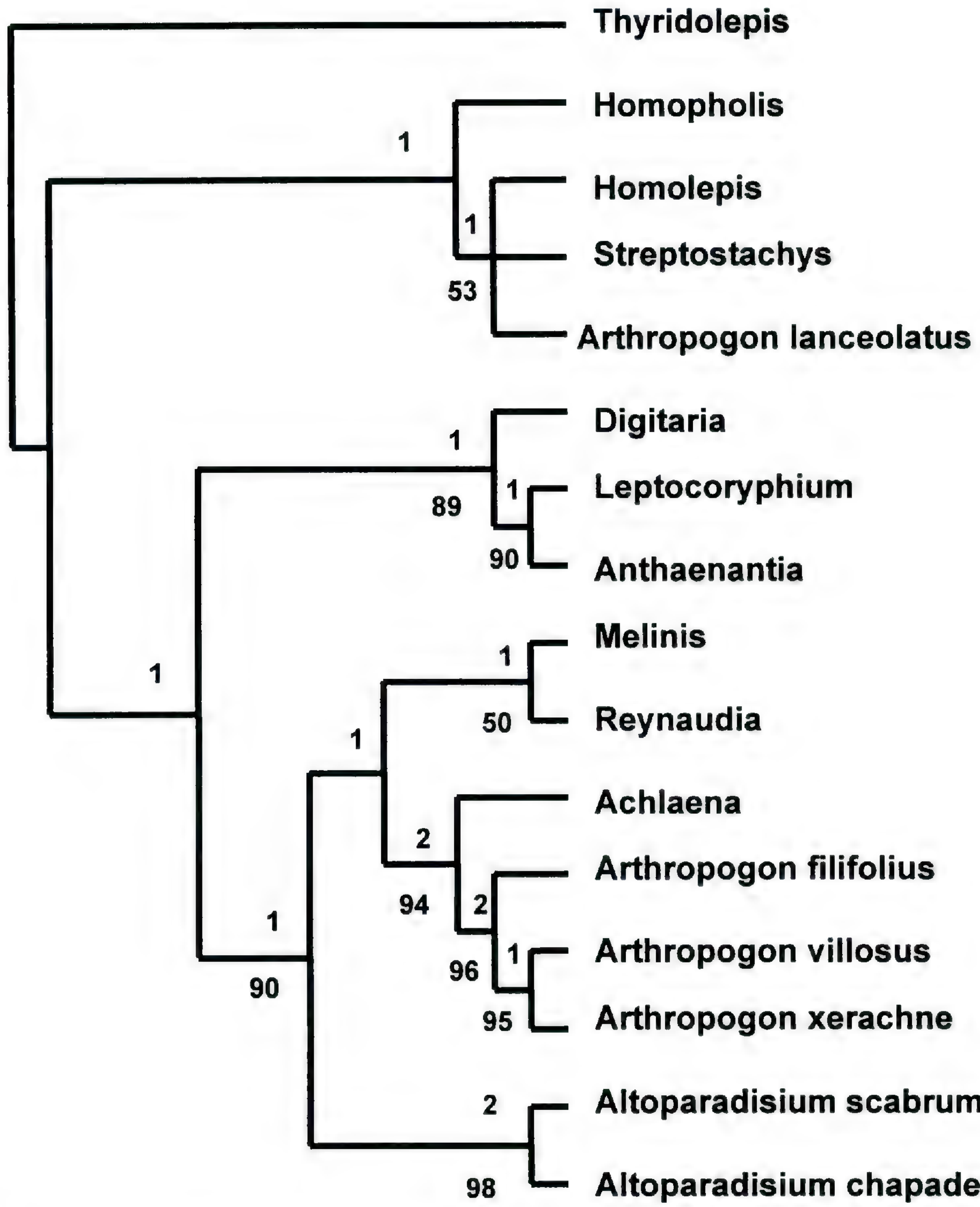


Figure 5. Strict consensus tree derived from the three equally most parsimonious trees. The upper numbers on each branch indicate the Bremer support values, while the lower numbers indicate bootstrap percentages.

strap value for this group is low (53). A similar topography was shown in a previous analysis (Zuloaga et al., 2000).

Altoparadisium chapadense and *A. scabrum* form a clade, supported by two synapomorphies: the relative development of the upper palea ¼ or less as long as the upper lemma (character 14), and the presence of intercostal grooves on the upper glume (character 22). This is clear support that these two species are congeneric, and for this reason we transfer *Arthropogon scaber* to *Altoparadisium.* Within this clade, *A. chapadense* is distinguished by the lower glume absent (character 5), upper lemma absent (character 13), upper palea absent (character 14), and awn of the upper glume twisted (character 31). In the initial scoring of the characters, it became evident that *Arthropogon scaber, A. rupestris,* and *A. bolivianus* could not be differentiated using the characters of the cladistic analysis. Consequently, we consider these three taxa to represent a single species. However, these taxa seem at least modally distinct in the degree of leaf blade infolding and scabrosity, as well as spikelet and inflorescence size. For this reason we do recognize them at the varietal level and make the necessary new combinations below.

The *Melinis–Reynaudia* clade, although poorly supported with a bootstrap percentage of only 50, is a sister to the *Achlaena piptostachya–Arthropogon* sensu stricto clade. The latter clade is sup-

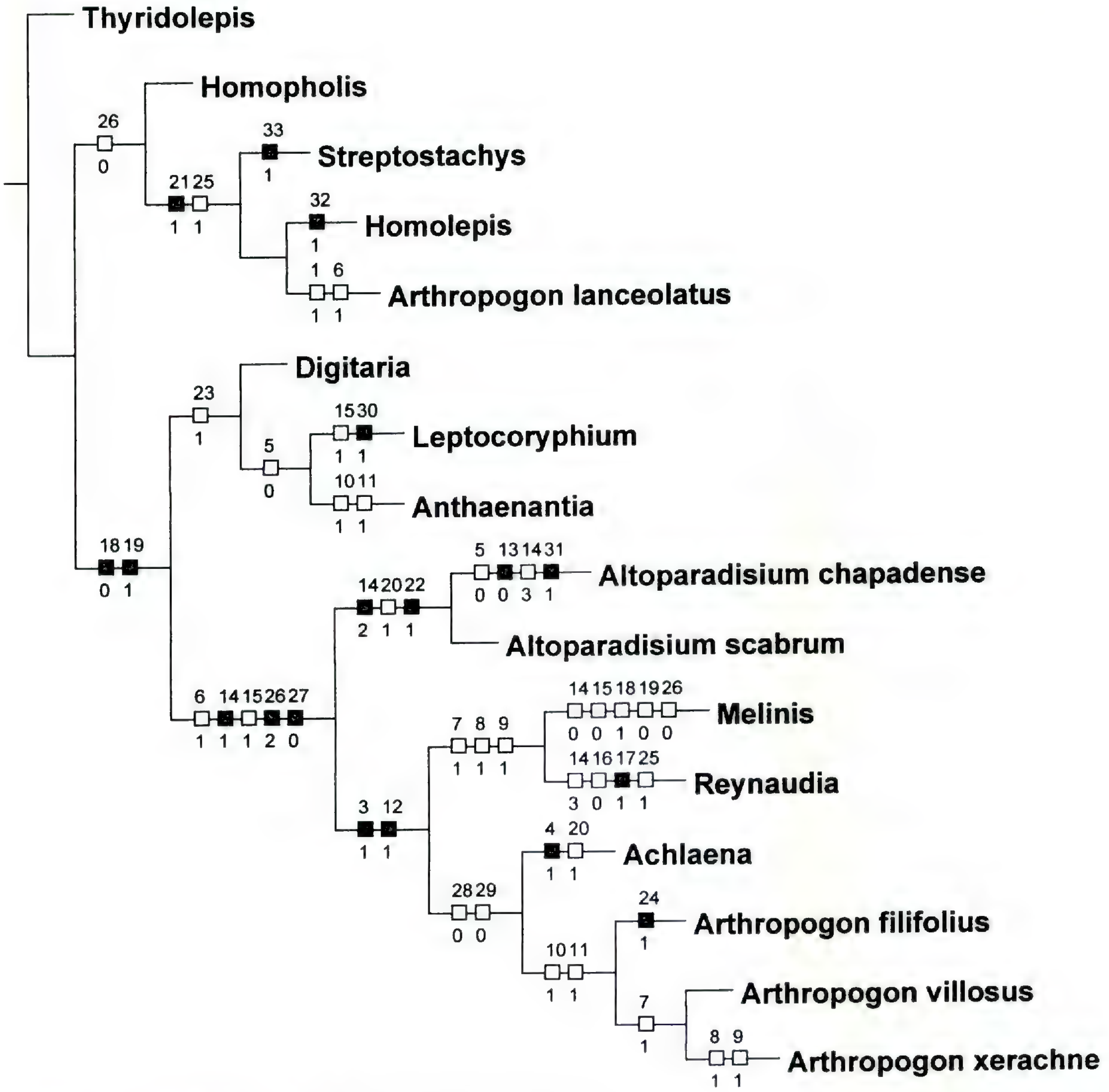


Figure 6. Cladogram of one of the three most parsimonious trees; the character numbers marked with the solid bars indicate synapomorphies, those with hollow bars homoplasious characters. L (length): 57; RI (retention index): 0.72; CI (consistency index): 0.52.

ported by two homoplasious characters, coriaceous upper glume (character 28), and coriaceous lower lemma (character 29), and is strongly supported with a bootstrap percentage of 94. Within this clade, *Arthropogon piptostachyus* is distinguished by two autapomorphies, the presence of a notable internode between the lower and upper glume (character 4), and the presence of distinctive Kranz cells (character 20). The clade formed of *A. filifolius–A. xerachne* is supported by two apomorphies, the presence of a palea (character 10) and lower flower (character 11), and it has strong bootstrap support (94). In addition to its morphological distinctiveness the Cuba-Jamaica endemic, *Achlaena piptostachya,* is geographically disjunct from the central Brazilian species of *Arthropogon* sensu stricto. These two factors combined give good support for the continued recognition of *Achlaena* as a separate genus.

In summary, as a result of this study we restrict *Arthropogon* to *A. xerachne, A. villosus,* and *A. filifolius*; *Arthropogon scaber* is transferred to the new genus *Altoparadisium*; *Arthropogon piptostachyus* is again recognized as *Achlaena piptostachya*; and finally, *Arthropogon lanceolatus* should be excluded from the genus *Arthropogon,* but its exact relationships and position in the tribe Paniceae remain unresolved. This can be explained by inspection of

the character distribution on the cladogram, which indicates there are relatively few nodes that are unambiguously supported by non-homoplasious characters. This is expressed statistically by the low consistency index, as well as by low bootstrap percentiles.

Taxonomic Treatment

History of *Arthropogon* and its Suprageneric Classification

The genus *Arthropogon* has a long and complex history. It was described by Nees (1829) on the basis of one species, *A. villosus*, from Brazil. Subsequently Hackel (1887) amplified the description of the genus and illustrated *A. villosus*. Ekman (1911), Pilger and Kuhlmann in Kuhlmann (1922), and more recently Filgueiras (1981, 1982, 1986, 1996) described new taxa in *Arthropogon*. Filgueiras (1982) revised the genus and has recognized eight species (Filgueiras, 1996).

The position of the genus in the Panicoideae has been controversial. Nees (1829), when describing it, included *Arthropogon* in the Saccharinae Griseb. Kunth (1833) and Steudel (1853–1855) included the genus in the tribe Andropogoneae Dumort. Bentham and Hooker (1883) and Hackel (1887) placed it in the tribe Tristegineae Benth. & Hook. f. Hitchcock (1936) included the genus in the Melinideae Hitchc., together with *Arundinella* Raddi and *Melinis* P. Beauv. Bews (1929) and Roshevits (1937) separated *Arundinella*, *Trichopterix* Chiov., *Danthoniopsis* Stapf, and other genera in the Arundinelleae, and included *Melinis* and *Arthropogon* in the Melinidinae. Pilger (1954) for the first time proposed the tribe Arthropogoneae Pilg. ex Butzin, and included *Arthropogon*, *Achlaena* Griseb., *Reynaudia*, and *Snowdenia* C. E. Hubb. However, his name remained invalid because he did not provide a Latin diagnosis or description. Butzin (1972) later validated the name and reviewed the taxonomy.

Tateoka (1963) indicated that of the four genera included by Pilger in the Arthropogoneae, *Snowdenia* does not pertain to this group. However, the other three genera are related and share the following characters: upper lemma membranous, glumes somewhat indurate, spikelets with lateral compression, and stigmas with terminal exsertion. Furthermore, based on anatomical characters, Tateoka (1963) suggested that *Arthropogon* could represent a taxon intermediate between the Arundinelleae and Paniceae.

Filgueiras (1982) illustrated and described the morphological and anatomical variation in *Arthropogon*. He classified it in the tribe Arthropogoneae, subtribe Arthropogoninae, together with *Reynaudia* and *Snowdenia*, following the criteria proposed by Pilger (1954): spikelets solitary; upper lemma thinly membranous, awnless and 1–3-nerved; upper palea thinly membranous, weakly nerved or nerveless.

Clayton and Renvoize (1986) included *Arthropogon* and *Reynaudia* in the subtribe Arthropogoninae and included *Snowdenia* in subtribe Cenchrinae Dumort. They noted the close affinity of *Snowdenia* with *Pennisetum* Rich. subsect. *Beckeropsis* (Fig. & De Not.) Hack. and hypothesized that *Snowdenia* was probably derived from it by suppression of the bristles subtending the spikelets. *Snowdenia* possesses dorsally compressed spikelets, a reduced lower glume, and has terminally exserted stigmas. When describing the genus, Hubbard (1929) grouped *Snowdenia* with *Acritochaete* Pilg. (subtribe Melinidinae of Clayton & Renvoize, 1986) because they have a similar inflorescence and spikelet morphology.

Grisebach (1866) described the genus *Achlaena* as monotypic and endemic to Cuba, based on *A. piptostachya*. Pilger (Hoehne & Pilger, 1922) transferred it to *Arthropogon*, and this has been generally accepted (Tateoka, 1963; Filgueiras, 1982; Clayton & Renvoize, 1986; Webster et al., 1989). However, Watson and Dallwitz (1992) recognized *Achlaena* at the generic level based on its restriction to Cuba and the following morphological characters: inflorescence open, spikelets compressed laterally, callus hairy, glumes 2 and narrow, and the upper lemma awnless and acuminate.

Brown (1977), based on anatomical characters, recognized the tribe Arthropogoneae and included *Achlaena*, *Arthropogon*, and *Reynaudia*. He emphasized that the relationship of *Snowdenia* (African) with the other three genera (American) is questionable because, following Tateoka (1963), *Snowdenia* has minute and membranous glumes, dorsally compressed spikelets, and stigmas exserted from the tip of the floret.

Webster et al. (1989) basically characterized *Arthropogon* by the presence of a differentiated callus at the base of the spikelet, spikelets laterally compressed, lower glume awned, and the upper lemma hyaline to membranous.

Key to the Genera *Altoparadisium*, *Achlaena*, and *Arthropogon*

1a. Spikelets dorsally compressed; upper palea absent or reduced; intercostal areas of the upper glume prominently sulcate ..... *Altoparadisium*
1b. Spikelets laterally compressed; upper palea well developed; intercostal areas of the upper glume flat.
  2a. Spikelets stipitate; distinctive Kranz cells present ..... *Achlaena*
  2b. Spikelets not stipitate; distinctive Kranz cells absent ..... *Arthropogon*

Altoparadisium Filg., Davidse, Zuloaga & Morrone, gen. nov. TYPE: *Altoparadisium chapadense* Filg., Davidse, Zuloaga & Morrone.

Hoc genus novum *Arthropogoni* Nees simile sed spiculis dorsaliter compressis, palea supera redacta vel nulla, nervis intercostalibus glumae superae manifeste sulcatis absimile.

Plantae perennes, caespitosae. Culmi erecti. Inflorescentia paniculata, contracta, terminalis. Spiculae solitariae vel binatae, anguste oblongo-lanceolatae, dorsaliter compressae; callus non manifestus sed pilis densis, verticillatis ornatus; spiculae 2–4 bracteis munitae; gluma inferna nulla vel setacea; gluma supera spiculam aequans, membranacea, 3–5-nervia, arista irregulariter tortili ornata; palea inferna nulla; flosculus infernus nullus lemmati infero redactus; lemma inferum membranaceum vel herbaceum, 3-nervium, nervus medianus piloso-hispidus, internervi sulcati; palea inferna nulla; flos inferus nullus; lemma superum hyalinum, 3-nervium vel nullum; palea supera redacta, ⅕ lemma aequans, hyalina sive nulla; flos unicus, bisexualis; lodiculae 2, conduplicatae; stamina 3; stigmata 2, purpurea, discreta. Caryopsis anguste oblongo-lanceolata, dorsaliter compressa, basibus stylorum persistentibus; hilum ellipticum ad oblanceolatum, atro-brunneum, ca. ⅕ caryopsidem aequans; embryo ca. ⅓ caryopsidem aequans.

Caespitose perennials. Culms erect; internodes hollow. Leaves primarily cauline; sheaths round on the back, the margins free; auricles absent; ligule a minute fringe of hairs; collar undifferentiated; blades linear-lanceolate. Inflorescence a contracted, terminal panicle; rachis glabrous, tough; nodes pilose; branches verticillate. Axillary inflorescences absent. Pedicels of unequal length. Spikelets paired or solitary, all alike, dorsally compressed, narrowly oblong-lanceolate in outline, disarticulating below the bracts and falling as a unit, with only one bisexual flower; callus undifferentiated except for a dense whorl of colorless hairs at the base of the spikelet; spikelet bracts 2 to 4; lower glume absent to setaceous; upper glume as long as the spikelet, embracing the lower lemma almost entirely, membranous, 3- to 5-nerved, the lateral nerves pilose-hispid or scabrous, the midnerve pilose-hispid toward the apex, projected into a straight or irregularly twisted awn; lower floret reduced to only a lemma; lower lemma membranous or herbaceous, 3-nerved, the midnerve prominent, pilose-hispid, the internerves sulcate, the margins hyaline and sharply folded at the lateral nerves, the apex pointed; lower palea absent; lower flower absent; upper lemma hyaline, 3-nerved, or absent; upper palea reduced, up to ⅕ as long as the upper lemma, hyaline, or absent; flower bisexual; lodicules 2, conduplicate; stamens 3; ovary glabrous; stigmas 2, separate, purple. Caryopsis narrowly oblong-lanceolate in outline, dorsally compressed, light brown, with persistent stylar bases, loosely fitting inside the glumes when fully developed; hilum elliptical to oblanceolate, dark brown, ca. ⅕ as long as the caryopsis; embryo about ⅓ as long as the caryopsis.

KEY TO THE SPECIES AND VARIETIES OF *ALTOPARADISIUM*

1a. Lower glume and upper lemma absent; upper palea absent; upper glume with a twisted awn ..... *A. chapadense*

1b. Lower glume and lemma present; upper palea present; upper glume with a straight awn.

2a. Leaf blades tightly involute ..... *A. scabrum* var. *bolivianum*

2b. Leaf blades flat.

3a. Spikelets 6–7 mm long; leaf blades scabrous; inflorescences 15–25 cm long ..... *A. scabrum* var. *scabrum*

3b. Spikelets 4–5 mm long; leaf blades smooth; inflorescences 7–9 cm long ..... *A. scabrum* var. *rupestre*

1. **Altoparadisium chapadense** Filg., Davidse, Zuloaga & Morrone, sp. nov. TYPE: Brazil. Goiás: Chapada dos Veadeiros, município of Alto Paraíso, ca. 26 km a oeste of Alto Paraíso, ca. 14°07′S–47°30′W, campo rupestre, estrada para Colinas do Sul Goiás, 5 Sep. 1994, *T. S. Filgueiras & M. L. Fonseca 2987* (holotype, IBGE!; isotypes, ICN!, K!, MO!, SI!, SP!, US!). Figure 1.

Altoparadisio scabro (Pilg. & Kuhlm.) Filg. et al. simile sed spiculis duabus tantum bracteis et aristis tortilibus differt.

Moderately caespitose, rhizomatous perennials. Rhizomes small, knotty, superficial; young rhizomes covered with cataphylls. Culms 75–80 cm tall, erect, some branched at around mid-height, others unbranched, with 6–9 elongated internodes; internodes 3–4.5 cm long, hollow, glabrous, straminеous; prophyll 2.5–4 cm long, strongly 2-keeled, ciliate along the keels, the margins hyaline, glabrous; nodes dark, pilose. Leaves cauline only, symmetrically distributed along the culm or congested at around mid-culm; sheaths round on the back, persistent, striate, glabrous, the margins free, hyaline, both glabrous or one margin ciliate; auricles absent; ligule a minute fringe of hairs, curved, the hairs 0.3–0.5 mm long, colorless, dense; hairs behind the ligular area 2–6 mm long, colorless, sometimes caducous in older leaves; collar undifferentiated, but seen in fresh material as a lighter or purplish area; blades 6–10 cm long and 3–8 mm wide, linear-lanceolate, glabrous on both surfaces but rough on the adaxial surface, the margins glabrous but rough, the apex subpungent to pungent; blades of the lower leaves caducous, the upper blades persistent. Peduncle 2–4.5 cm long, from

partially enclosed in the flag leaf to exserted, glabrous. Inflorescence 6.5–14.5 cm long and 1.5–3.5 cm wide, a contracted, terminal panicle, oblong-lanceolate, pointed at the apex, light-colored, smooth; rachis glabrous, tough; ramification verticillate; nodes pilose, the hairs 1–3 mm long, colorless; branches 4–9 mm long, 2–8 per node, glabrous. Axillary inflorescences absent. Pedicels 1–3 mm long, of unequal length, with a few colorless hairs at the base, the hairs 1–3 mm long, the apex of the pedicels discoid, surrounded by a crown of colorless hairs, the hairs 2–4 mm long. Spikelets paired or solitary, all alike, dorsally compressed, smooth, light-colored, 3–3.2 mm long (exclusive of awn), narrowly oblong-lanceolate in outline, disarticulating below the bracts and falling as a unit, with only one bisexual flower; callus undifferentiated but the hairs at the base of the spikelet 1–1.5 mm long, dense, forming a crown, colorless; spikelet bracts 2 [here interpreted as an upper glume and lower lemma—see discussion]; lower glume absent; upper glume as long as the spikelet, embracing the lower lemma almost entirely, membranous, the margins flat but slightly curved around the lateral nerves of the upper lemma in closed spikelets and fitting into the internervial grooves of the lower lemma, 3- to 5-nerved, the lateral nerves pilose-hispid, the midnerve pilose-hispid toward the apex, projected into a straight or irregularly twisted awn; awn 18–26 mm long, antrorsely barbed, highly hygroscopic; lower palea absent; lower floret reduced to only a lemma; lower lemma 3–3.1 mm long, thin, membranous, 3-nerved, the midnerve prominent, pilose-hispid, the internerves shallowly sulcate, the margins hyaline and sharply folded at the lateral nerves, the apex pointed, ciliate; lower palea absent; lower flower absent; upper floret reduced to only the flower; upper lemma absent; upper palea absent; flower bisexual; lodicules 2, conduplicate; stamens 3, the anthers 1.8–2.1 mm long, purple; ovary 0.5–0.8 mm long, glabrous; stigmas 2, separate, purple. Caryopis 1.8–1.9 mm long, narrowly oblong-lanceolate in outline, dorsally compressed, light brown, with persistent stylar bases, loosely fitting inside the glumes when fully developed; hilum elliptical to oblanceolate, dark brown, ca. $\frac{1}{5}$ as long as the caryopsis; embryo ca. $\frac{1}{3}$ as long as the caryopsis.

TRANSVERSE SECTION OF LEAF BLADE (FIG. 2)

Leaf outline. Leaf blade expanded, undulating gently on the adaxial surface, the undulations slightly more pronounced in association with first-order vascular bundles resulting in shallow adaxial furrows on either side, without abaxial ribs.

Midrib. Consisting of a single first-order vascular bundle similar in structure to the lateral first-order vascular bundles with no additional associated parenchyma or keel.

Vascular bundle arrangement. 3(or 4) third-order bundles between the first-order bundles; 3 or 4 fourth-order bundles, represented by solitary distinctive Kranz cells between third-order bundles or between first-order and third-order bundles; all bundles more or less centrally located, but first-order and third-order bundles slightly off-center adaxially and fourth-order bundles slightly off-center abaxially.

Vascular bundle description. Third-order bundles vertically elongated and often nearly dumbbell-shaped in outline, with xylem and phloem indistinguishable. First-order bundles vertically elongated in outline. Metaxylem vessels narrow, that is, parenchyma sheath cells wider than the vessel elements; no lysigenous cavity and enlarged protoxylem element present.

Vascular bundle sheaths. In third-order bundles outer parenchyma sheath vertically elongated, complete, composed of 9 to 11 cells, without adaxial or abaxial extensions, with a single large colorless cell located on each side of the sheath in about the center of the blade with the adjoining one or two parenchyma bundle sheaths usually distinctively smaller than the majority of the parenchyma bundle sheath cells.

In first-order bundles outer parenchyma sheath vertically elongated, with or without a slight adaxial interruption and with a wide abaxial interruption, composed of 10 to 13 cells, with a single, large colorless cell similar to those of the third-order bundles on each side of the sheath; parenchyma sheath cells distinct from the chlorenchyma cells with the radial and inner tangential walls straight and the outer tangential walls somewhat rounded, all cells equally thickened.

Sclerenchyma girders. Adaxial sclerenchyma girders associated with first- and second-order bundles, narrowing toward the bundles: girders never associated with fourth-order bundles; abaxial sclerenchyma girders associated with all first- and second-order bundles, well developed, narrowing toward the bundle.

Chlorenchyma. Not distinctly radiate, continuous between the epidermides and between the bundles; without arm cells, fusoid cells, lacunae, or colorless cells.

Adaxial epidermal cells. Groups of bulliform cells present only over groups of fourth-order bun-

dles, not over third- or first-order bundles, fan-shaped, situated at the base of slight furrows, the central cell ca. ⅓ as wide as the leaf thickness, the epidermal cells with the outer tangential walls slightly concave and a thin, continuous cuticle; macrohairs and papillae not present; prickles in both the costal and intercostal zones.

Abaxial epidermal cells. Bulliform cells not present; all cells with a continuous, thick cuticle, ca. twice as thick as the adaxial cuticle. The radial cell walls somewhat thickened; papillae absent; prickles absent.

ADAXIAL EPIDERMIS IN SURFACE VIEW (FIG. 3B)

Intercostal long cells. Elongated 3–5 times longer than wide, rectangular in shape; cell walls moderately thickened; anticlinal cell walls vertical; horizontal anticlinal walls moderately undulate; cell shape relatively constant, cell size variable in single files. Single short cells usually present between 75% or more of successive long cells. Stomata: absent. Papillae: absent. Prickles: numerous in both the intercostal and costal zones. Microhairs: absent. Silica bodies: varying from squarish and angular with irregular outlines to cuboid, more or less squarish with rounded corners.

ABAXIAL EPIDERMIS IN SURFACE VIEW (FIG. 3A, C, D)

Intercostal long cells. Elongated 2.5–3.7 times longer than wide, rectangular in shape, cell walls slightly thickened, anticlinal vertical cell walls angled, horizontal cell walls moderately to slightly undulate; cell shape relatively constant; cell size variable in a single file; pairs or single short cells sometimes present between successive long cells. Stomata: Subsidiary cells low dome-shaped to low-triangular-shaped; four rows of stomata in each intercostal zone most common, usually in double rows adjoining the costal zones; intercostal long cells 1.5–3 times longer than wide, one or two interstomal cells between successive stomata of a row. Single short cells present between costal long cells.

Papillae absent. Prickle hairs present between both costal and intercostal successive long cells. Microhairs infrequent, bicellular, but only the basal cell visible in our preparations [microhairs present on both the upper glume and lower lemma, bicellular, elongated, the basal ca. ½ as long as the distal cell, less than twice as long as wide, with thicker walls than the distal cell, the distal cell more than twice the width, the apex sharply pointed] (Fig. 3C, D).

Silica bodies equidimensional, round to squarish with irregular outlines.

NOTES

The soft texture of the inflorescence and spikelets is intriguing since the caryopsis seems to get almost no protection from the flowering bracts. The most common pattern in the Paniceae in general seems to be that when one bract is missing, the remaining bracts become hardened and protective in function, so as to compensate for the missing structure. *Altoparadisium chapadense* seems to be an outstanding exception to this rule. The bracts here are scarcely hard enough to protect the flower and later the caryopsis. In the later stages of development, the caryopsis remains rather loose inside the bracts. Where does protection for the growing seed and embryo come from? Certainly not from the upper lemma and upper palea, since they are completely lacking. The stiff blades may, to some extent, provide some indirect protection by discouraging the approach of large herbivores, but surely not small herbivores, such as insects, which appear to play an important role in the predation of grass leaves and fruits in the Cerrado vegetation (Filgueiras, 1989).

The generic name alludes to the town of Alto Paraíso in whose municipality the original population was discovered. The specific epithet refers to the Chapada dos Veadeiros, and at the same time honors the Chapadense family of Goiás, Brazil, whose members have been supportive and interested in the work of one us (TSF).

Ecology. Flowering plants in the field have a rather bambusoid appearance, with stiff, congested leaves around mid-culm level. Non-flowering plants have normal-looking blades. The branching of the culms seems to vary within the same clump; some plants in a given clump have branched culms whereas others do not.

The original population discovered was found growing in a campo rupestre habitat, on fine white sand and also on rocks. The soil, very shallow, stony, and dry, was a mixture of gravel and sand. Four groups of plants made up this population, with 2, 5, 15, and 20 clumps respectively. Each of these clumps had from 2 to 12 flowering culms. The groups of plants were situated 50 to 100 m from each other, and the entire population covered an area of approximately 200 m^2. It is very likely that other populations exist in the area, but none were located, although at that time of the year (September) the light-colored inflorescences of *A. chapa-*

dense stand out against the dry, brown colors of the campo rupestre landscape.

Plants of *A. chapadense* were growing in association with the following species: *Axonopus brasiliensis* (Spreng.) Kuhlm., *Echinolaena inflexa* (Poir.) Chase (the campo rupestre form), *Elionurus muticus* (Spreng.) Kuntze, *Lagenocarpus* sp., *Paspalum polyphyllum* Nees, etc. Except for *E. inflexa,* which flowers almost year-round, no plants of these species were in flower on 5 September 1994. Although the plants were collected during the peak of the flowering period for this population, the leaves had an old, dried out appearance and some had dropped their blades. Almost all the blades displayed some characteristic, irregularly shaped dark spots, suggestive of fungal infection.

During a second trip to the same locality 78 days after the first (24 November 1994), after the first rains had already fallen, it was found that the seeds of *A. chapadense* had long since been dispersed and new growth could be observed at the base of the clumps. At that time, three live plants of *A. chapadense* were taken from the wild and planted at the Reserva Ecológica do IBGE (ca. 15°57′S, 47°52′W), a conservation unit in the Distrito Federal, Brazil, where they are growing well. Two of these plants were planted directly in the ground, and one is being cultivated in a clay pot. In both cases the soil is red latosol, without the use of organic or chemical fertilizers. The plants under cultivation have not developed the above-mentioned dark spots on the blades. Since transplantation, all these plants have flowered four times, always in the last week of August through September. As of September 2000, no seedlings of *A. chapadense* have been observed in the vicinity of the mother clumps under cultivation.

In August 1997 a second population of *A. chapadense* was discovered in the municipality of Niquelândia, state of Goiás, about 145 km west of Alto Paraíso, on the grounds of a private ecological preserve (owned by a local mining firm, "Companhia de Níquel Tocantins") called "Fazenda Engenho." The plants in this population grow in an area ecologically similar to the plants in the Alto Paraíso population. According to the collectors of the plants from the latter population, the plants grew in scattered clumps in an area of approximately 100 m².

Paratype. BRAZIL. **Goiás:** Niquelândia, Fazenda Engenho, Lama Preta, ca. 11 km Niquelândia, 14°39′08″S, 48°26′23″W, 14 Aug. 1997, *F. Oliveira et al. 839* (IBGE, MEXU, VEN).

2. Altoparadisium scabrum (Pilg. & Kuhlm.) Filg., Davidse, Zuloaga & Morrone, comb. nov. Basionym: *Arthropogon scaber* Pilg. & Kuhlm., Comm. Lin. Telegr., Bot. 67(anex. 5): 37, pl. 2. 3 Mar. 1922. TYPE: Brazil. Mato Grosso: Cáceres, Agua Quente, *Hoehne 2015* (holotype, R!). See Filgueiras (1982) for further information about the problems with the labeling of the type collection. Figure 7.

Arthropogon scaber Kuhlm. & Pilg., Revista Mus. Paul. Univ. São Paulo 13: 1249. 24 Dec. 1922, nom. illeg. hom. TYPE: Brazil. Mato Grosso: nos campos de Juruena, etc., June 1909, *Hoehne 2016* (holotype, B; isotype, US-865392 (fragm. ex B)!).

2a. Altoparadisium scabrum var. **scabrum**

2b. Altoparadisium scabrum (Pilg. & Kuhlm.) Filg., Davidse, Zuloaga & Morrone var. **bolivianum** (Filg.) Filg., Davidse, Zuloaga & Morrone, comb. nov. Basionym: *Arthropogon bolivianus* Filg., Brittonia 38: 71. 1986. TYPE: Bolivia. Santa Cruz: near the Arches, 5 km N of Santiago of Chiquitos, 700 m, 2 Sep. 1942, *Cutler 7023* (holotype, US-2013303!).

2c. Altoparadisium scabrum (Pilg. & Kuhlm.) Filg., Davidse, Zuloaga & Morrone var. **rupestre** (Filg.) Filg., Davidse, Zuloaga & Morrone, comb. nov. Basionym: *Arthropogon rupestris* Filg., Nordic J. Bot. 16: 69, f. 1. 1996. TYPE: Brazil. Goiás: Serra do Caiapó, 17°12′S, 51°47′W, ca. 50 km S of Caiapônia on road to Jataí, 26 Oct. 1964, *Irwin & Soderstrom 7390* (holotype, SP!; isotype, US!).

Arthropogon Nees, Fl. Bras. Enum. Pl. 2: 319. 1829. TYPE: *Arthropogon villosus* Nees.

KEY TO THE SPECIES OF *ARTHROPOGON*

1a. Upper glume with the apex entire; leaf blades cylindrical ..... *A. filifolius*
1b. Upper glume with the apex bilobed; leaf blades flat.
2a. Lower lemma awned, the apex bilobed ..... *A. xerachne*
2b. Lower lemma muticous, the apex entire ..... *A. villosus*

1. Arthropogon filifolius Filg., Bradea 3(21): 151, f. 1. 1981. TYPE: Brazil. Distrito Federal: Cabeça do Veado, capim de espiguetas aristadas, crescendo em brejo recém queimado, 15 Sep. 1980, *T. S. Filgueiras 671* (holotype, IBGE!; isotypes, R!, RB!, SP!, US-2946634!).

Known only from Brazil (Goiás, Minas Gerais, and the Distrito Federal).

Additional specimen examined. BRAZIL. **Minas Gerais:** Uberlândia, just N of town, left side RR, 850–870 m, 15 Mar. 1930, *Chase 11261 1/2* (US).

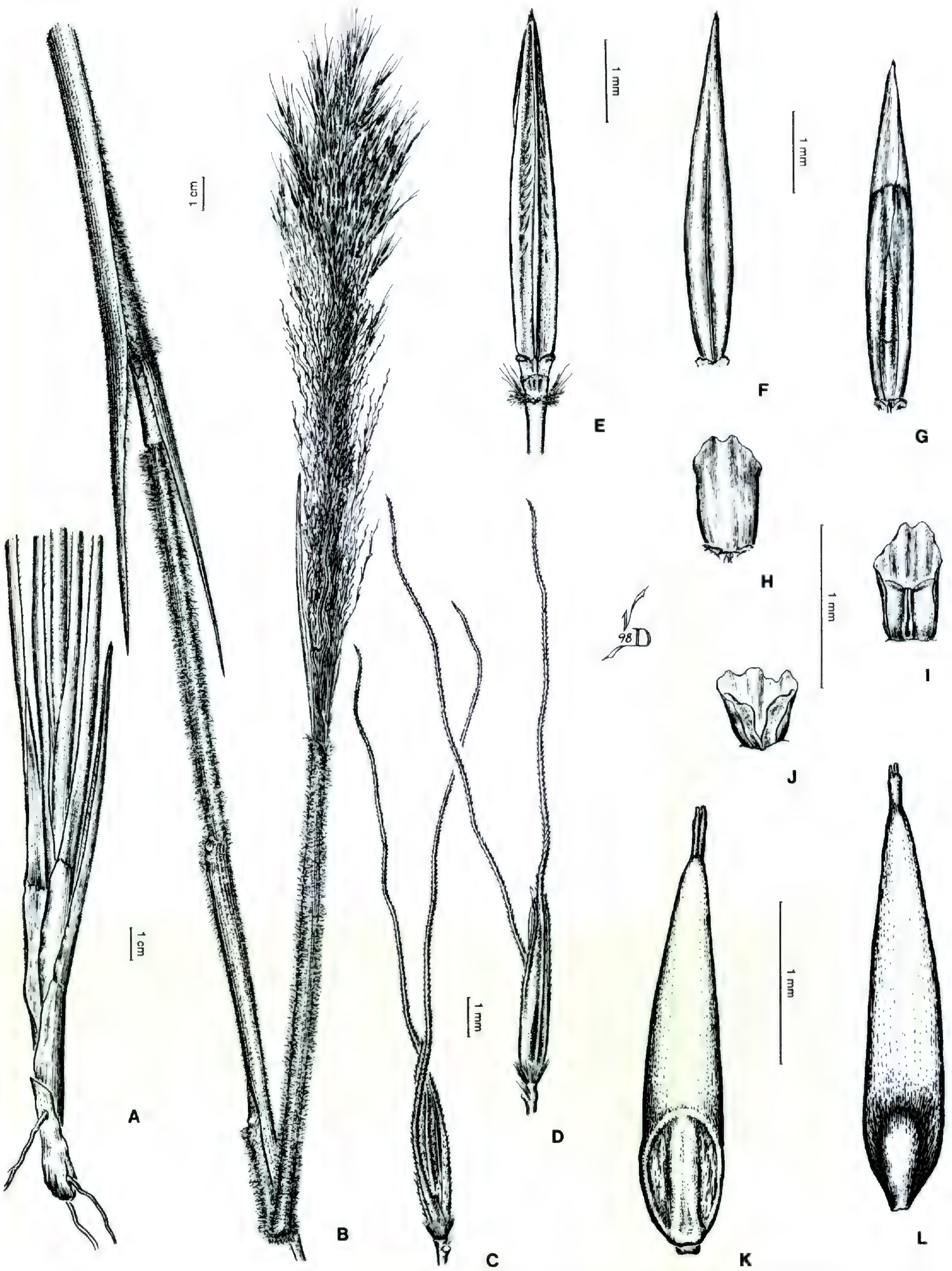


Figure 7. *Altoparadisium scabrum* var. *scabrum* (based on *Kuhlmann 1853*, US). —A. Base of the plant. —B. Habit, upper portion of a culm with a terminal inflorescence. —C. Spikelet seen from the side of the lower glume with hairs at the apex of the pedicel and the base of the callus. —D. Spikelet seen from the side of the upper glume. —E. Lower floret seen from the side of the lower lemma, with prominent nerves, and base of the spikelet with callus. —F. Upper lemma, dorsal view. —G. Upper lemma, ventral view, with stamens and reduced palea at the base. —H. Upper palea, dorsal view. —I. Upper palea and lodicules, ventral view. —J. Upper palea, ventral view. —K. Caryopsis, embryo side. —L. Caryopsis, hilum side. All drawings by Vladimiro Dudás.

2. **Arthropogon villosus** Nees, Fl. Bras. Enum. Pl. 2: 320. 1829. TYPE: Brazil. "Habitat in campis altis Chapada of Paranan provinciae Minarum versus Goyaz, et ad Rio Fermoso," *Martius s.n.* (holotype, M!; isotype fragment, US-865393!).

Known from Brazil (Bahia, Distrito Federal, Goiás, Mato Grosso, Minas Gerais, Paraná, and São Paulo), and Bolivia (Santa Cruz).

Additional specimens examined. BRAZIL. **Bahia:** 22 km S of the Rio Galheirao along Hwy. BR-020, 860 m, 8 Apr. 1976, *Davidse et al. 12222* (US), *12222A* (MO). **Distrito Federal:** burned-over campo and cerrado, summit of Chapada da Contagem, ca. 10 km NE of Brasília, 1000 m, 11 Sep. 1965, *Irwin et al. 8174* (MO); cerrado, gallery forest and adjacent burned-over cerrado, Córrego Urubu, wet slopes of Chapada da Contagem, ca. 10 km E of Brasília, 1000 m, 14 Sep. 1965, *Irwin et al. 8309* (MO); cerrado ca. 20 km S of Brasília on road to Goiânia, near Rio Melchoir, 1125 m, 25 Sep. 1965, *Irwin et al. 8661* (MO). **Mato Grosso:** Braco, Rio Arinos, 26 Sep. 1943, *Baldwin 3041* (US). **Minas Gerais:** Lavras, 4 Mar. 1925, *Chase 8844* (MO); Serra do Cipó, Km 106-90 (110 km NE of Belo Horizonte), 800 m, 28 Mar.–1 Apr. 1925, *Chase 9283* (MO); S. Vicente, 10 Nov. 1956, *Macedo 2711* (MO, US). **Goiás:** Uruaçu, Fazenda Macaco, margem esquerda do córrego vermelho, 6 Oct. 1992, *Pinheiro C. et al. II 0041* (MO); hwy. between Planaltina and São Gabriel de Goiás ca. 5 km S of São Gabriel, 29 Nov. 1988, *Kral et al. 75720* (MO). **Paraná:** ad Morungava praedium pr. Itararé oppositum in campo, 720 m, 7 Dec. 1915, *Dusén 17406* (MO). BOLIVIA. **Santa Cruz:** Ñuflo de Chavez, Concepción, 500 m, 7 Aug. 1985, *Killeen 1112* (MO); Ñuflo de Chavez, Est. San Josecito, 2 km NE of Concepción, 500 m, 7 Sep. 1985, *Killeen 1178* (MO).

3. **Arthropogon xerachne** Ekman, Ark. Bot. 10(17): 9, pl. 1, f. 4, pl. 6, f. 4. 1911. TYPE: Brazil. São Paulo: S. José dos Campos, in campo, 1910, *Löfgren 243* (lectotype, here designated, S!; isolectotype, US-3168439 fragment!). This specimen is chosen as the lectotype because it agrees perfectly with the description, and because it has the most detailed locality data.

Known from Brazil (Paraná and São Paulo).

Additional specimens examined. BRAZIL. **Paraná:** without locality, 23 June 1910, *Dusén 10002* ([syntypes]S, US-3168438 fragment); Jaguariahyva, in campo cerrado, 730 m, 30 Nov. 1915, *Dusén 17367* (SI), 22 Oct. 1910, *Dusén 10748* (MO). **São Paulo:** Mun. S. José dos Campos, 6.8–7.2 km ao SSE em linha reta da praça principal of São José dos Campos, 12 Sep. 1962, *Mimura 563* (US); Campinas, 12 Dec. 1940, *Viegas & Lima s.n.* (US-1764757).

Achlaena Griseb., Cat. Pl. Cub. 228. 1886. TYPE: *Achlaena piptostachya* Griseb.

1. **Achlaena piptostachya** Griseb., Cat. Pl. Cuba: 229. 1866. *Arthropogon piptostachyus* (Griseb.) Pilg., Revista Mus. Paul. Univ. São Paulo 13: 1250. 1922. *Arthropogon piptostachyus* (Griseb.) Tateoka, Bot. Mag. (Tokyo) 76(902): 290. 1963, isonym. TYPE: Cuba. Without locality, *Wright 3487* (holotype?; isotypes, NY!, US-927103!, US-75310 fragment!).

Known from Cuba and Jamaica.

Additional specimens examined. CUBA. Río Piloto, Sierra de Nipe, Oriente, savannas on eruptive rock, 8 Sep. 1922, *Ekman Amer. Gr. Nat. Herb. 800* (MO). JAMAICA. **Hanover:** Ashkenish to Dophin Head, pasture on clay soil, 8 Sep. 1960, *Adams 7952* (MO).

Literature Cited

Bentham, G. & J. D. Hooker. 1883. Gramineae. *In* Genera Plantarum 3(2): 1074–1215. L. Reeve, London.

Bews, J. W. 1929. The World's Grasses. Longmans, Green, London.

Blake, S. T. 1958. New criteria for distinguishing genera allied to *Panicum* (Gramineae). Proc. Roy. Soc. Queensland 70: 15–19.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10: 295–304.

Brown, W. V. 1977. The Kranz syndrome and its subtypes in grass systematics. Mem. Torrey Bot. Club 23: 1–97.

Burkart, A. 1969. Gramíneas. Fl. Il. Entre Ríos. Colecc. Ci. Inst. Nac. Tecnol. Agropecu. 6(2): 1–551.

Butzin, F. 1972. Zur Nomenklatur und Taxonomie der Arthropogoneae (Gramineae, Panicoideae). Willdenowia 6: 511–517.

Chase, A. 1911. Notes on genera of Paniceae. IV. Proc. Biol. Soc. Wash. 24: 103–160.

Cialdella, A. M. & A. S. Vega. 1996. Estudios sobre la variación estructural de las espiguillas en genéros de la tribu Paniceae (Poaceae). Darwiniana 34: 173–182.

Clayton, W. D. & S. A. Renvoize. 1986. Genera graminum. Grasses of the world. Kew Bull., Addit. Ser. 13: 1–389.

Cochrane, T. T., S. Nichez, L. G. Azevedo, J. A. Porras & C. L. Garver. 1985. Land in Tropical America. Cali, CIAT- MBRAPA-CPAC.

Cutler, D. 1978. Applied Plant Anatomy. Academic Press, London.

Dengler, N. G., R. E. Dengler & P. W. Hattersley. 1985. Differing ontogenetic origins of PCR ("Kranz") sheaths in leaf blades of C_4 grasses (Poaceae). Amer. J. Bot. 72: 284–302.

Ekman, E. L. 1911. Neue brasilianischer Gräser. Ark. Bot. 10(17): 1–15.

Ellis, R. P. 1976. A procedure for standardizing comparative leaf blade anatomy in the Poaceae. I. The leaf blade as viewed in transverse section. Bothalia 12: 65–109.

———. 1977. Distribution of the Kranz syndrome in the Southern African Eragrostideae and Panicoideae ac-

cording to bundle sheath anatomy and cytology. Agroplantae 9: 73–110.

———. 1979. A procedure for standardizing comparative leaf blade anatomy in the Poaceae. II. The epidermis as seen in surface view. Bothalia 12: 641–672.

———. 1987. A review of comparative leaf blade anatomy in the systematics of the Poaceae: The past twenty-five years. Pp. 3–10 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

———. 1988. Leaf anatomy and systematics of *Panicum* (Poaceae: Panicoideae) in southern Africa. Monogr. Syst. Bot. Missouri Bot. Gard. 25: 129–156.

Farris. J. S. 1988. Hennig86. Reference, version 1.5. Port Jefferson Station. J. S. Farris, New York.

———. 1989. The retention index and the rescaled consistency index. Cladistics 5: 417–419.

Felfili, J. A., T. S. Filgueiras, M. Haridasan, M. C. Silva Jr., R. C. Mendonça & A. V. Rezende. 1994. Projeto biogeografia do bioma Cerrado: vegetação e solos. Cadernos de Geociências, 12: 75–166.

———, M. C. Silva Jr., A. V. Rezende, P. E. Nogueira, B. M. T. Walter & M. A. Silva. 1997. Composição florística e fitossociológica do cerrado na Chapada dos Veadeiros. Pp. 6–11 *in* L. Leite & C. H. Sato (editors), Contribuição ao Conhecimento Ecológico do Cerrado. Brasília, Editora da Universidade de Brasília.

Felsenstein, J. 1985. Confidence limits on phylogenetics: An approach using the bootstrap. Evolution 39: 783–791.

Filgueiras, T. S. 1981. Species duae gramineárum Brasiliae novae. Bradea 3: 151–155.

———. 1982. Taxonomia e distribuição de *Arthropogon* Nees (Gramineae). Bradea 3: 303–322.

———. 1986. A new species of *Arthropogon* (Gramineae: Arthropogoneae) and a revised key for the genus. Brittonia 38: 71–72.

———. 1989. Gramíneas e herbívoros considerações sobre o processo de coevolução adaptativa. Ciência e Cultura 41: 158–162.

———. 1996. *Arthropogon rupestris* (Poaceae: Arthopogoneae) a new species from the Brazilian cerrado vegetation and a revised key for the genus. Nordic J. Bot. 16: 69–72.

——— & F. O. Zuloaga. 1999. A new *Triraphis* (Poaceae: Eragrostideae) from Brazil: First record of a native species in the New World. Novon 9: 36–42.

Goloboff, P. A. 1993. NONA versión 1.8 (32 bit). Programa y documentación distribuida por el autor. Tucumán, Argentina.

Grisebach, A. 1866. Catalogus Plantarum Cubensium. Leipzig.

Hackel, E. 1887. Gramineae. *In* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien 2(2): 1–97. Engelmann, Leipzig.

Hattersley, P. W. & L. Watson. 1975. Anatomical parameters for predicting photosynthetic pathways of grass leaves: The "maximum lateral cell count" and the "maximum cells distant count." Phytomorphology 25: 325–333.

——— & ———. 1976. C_4 grasses: An anatomical criterion for distinguishing between NADP-Malic enzyme species and PCK or NAD-Malic enzyme species. Austral. J. Bot. 24: 297–308.

——— & ———. 1992. Diversification of photosynthesis. Pp. 38–116 *in* G. P. Chapman (editor), Grass Evolution and Domestication. Cambridge Univ. Press, Cambridge.

Hitchcock, A. S. 1936. Manual of the Grasses of the West Indies. United States Department of Agriculture Miscellaneous Publication 243: 1–439.

Hoehne, F. C. & R. Pilger. 1922. Novidades da flora mattogrossense e do Hervario da Comissão Rondon. Revista Mus. Paul. Univ. São Paulo 13: 1247–1254.

Hsu, C. C. 1965. The classification of *Panicum* (Gramineae) and its allies, with special reference to the characters of lodicule, style-base and lemma. J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 9: 43–150.

Hubbard, C. E. 1929. Notes on African grasses, VIII. *Snowdenia*, a new genus from Uganda. Bull. Misc. Inform. Kew 1929: 28–31.

Jacques-Félix, H. 1962. Les Graminées (Poaceae) d'Afrique Tropicale, I: Généralités classification, description des genres. Institut des Recherches Agronomiques Tropicales et des Cultures Vivrières, Bulletin Scientifique (Paris) 8: i–xi, 1–345.

Judziewicz, E. 1990. Family 187. Poaceae. *In* A. R. A. Görts-van Rijn (editor), Flora of the Guianas. Series A: Phanerogams. Koeltz Scientific Publications, Koenigstein, Germany.

Kellogg, E. A. & C. S. Campbell. 1987. Phylogenetic analyses of the Gramineae. Pp. 310–322 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

——— & L. Watson. 1993. Phylogenetic studies of a large data set. I. Bambusoideae, Andropogonodae, and Pooideae (Gramineae). Bot. Rev. 59: 273–340.

Killeen, T. & L. G. Clark. 1986. Fusoid-like cells in *Panicum* section *Laxa* (Poaceae: Panicoideae). Amer. J. Bot. 73: 771.

Kluge, A. G. & J. S. Farris. 1969. Quantitative phyletics and evolution of anurans. Syst. Zool. 18: 1–32.

Kuhlmann, J. G. 1922. Gramineas. Comm. Lin. Telegr., Bot. 67(Bot. 11): 1–95, t. 1–6.

Kunth, C. S. 1833. Enumeratio Plantarum . . . Agrostographia Synoptica . . . Supplement to Vol. 1. J. G. Cottae. Stuttgart, Tübingen.

Metcalfe, C. R. 1960. Anatomy of the Monocotyledons, Vol. 1: Gramineae. Clarendon Press, Oxford.

Morrone, O. & F. O. Zuloaga. 1991. Revisión del género *Streptostachys* (Poaceae–Panicoideae), su posición sistemática dentro de la tribu Paniceae. Ann. Missouri Bot. Gard. 78: 359–376.

———, ——— & E. Carbonó. 1995. Revisión del grupo *Racemosa* del género *Paspalum* (Poaceae: Panicoideae: Paniceae). Ann. Missouri Bot. Gard. 82: 82–116.

Munhoz, C. B. R. & C. E. B. Proença. 1998. Composição florística do município de Alto Paraíso na Chapada dos Veadeiros. Bol. Herb. Ezechias Paulo Heringer 3: 102–150.

Nees von Esenbeck, C. G. D. 1829. Flora Brasiliensis seu Enumeratio . . . , C. F. D. von Martius (editor), 2(1): 1–608.

Nicora, E. G. & E. Rúgolo de Agrasar. 1987. Los Géneros de Gramíneas de América Austral. Hemisferio Sur, Buenos Aires.

Nixon, K. C. 1993. Clados, version 1.38 [32 bit] beta version. Computer program distributed by the author.

Pilger, R. 1954. Das System der Gramineae. Bot. Jahrb. Syst. 76: 281–384.

Reger, B. J. & I. E. Yates. 1979. Distribution of photosynthetic enzymes between mesophyll specialized

chlorenchyma and bundle sheath cells of *Arundinella hirta*. Pl. Physiol. 63: 209–212.
Renvoize, S. A. 1982a. A survey of leaf blade anatomy in grasses II. Arundinelleae. Kew Bull. 37: 489–495.
———. 1982b. A survey of leaf blade anatomy in grasses III. Garnotieae. Kew Bull. 37: 497–500.
———. 1987. A survey of leaf blade anatomy in grasses XI. Paniceae. Kew Bull. 42: 739–768.
———. 1998. Gramíneas de Bolivia. Continental Printing, Belgica.
Rosengurtt, B., B. R. Arrillaga de Maffei & P. Izaguirre de Artucio. 1970. Gramíneas Uruguayas. Publicaciones de la Universidad de la República, Montevideo.
Roshevits, R. Y. 1937. Zlaki. Vvedeniye v isucheniye kormovykh i khlebnykh zlakov [Grasses. An Introduction to the Study of Fodder and Cereal Grasses]. Moscow, Leningrad.
Sánchez, E. & M. Arriaga. 1989. El síndrome de Kranz en Arundinelleae (Panicoideae–Poaceae) de la flora sudamericana. Parodiana 5: 261–277.
———, M. O. Arriaga & R. P. Ellis. 1989. Kranz distinctive cells in the culm of *Arundinella* (Arundinelleae; Panicoideae; Poaceae). Bothalia 19: 45–52.
Sendulsky, T., T. S. Filgueiras & A. G. Burman. 1987. Fruits, embryos and seedlings. Pp. 31–36 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.
Smith, B. N. & W. V. Brown. 1973. The Kranz syndrome in the Gramineae as indicated by carbon isotopic ratios. Amer. J. Bot. 60: 505–513.
Soderstrom, T. R. & R. P. Ellis. 1987. The position of bamboo genera and allies in a system of grass classification. Pp. 225–238 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press. Washington, D.C.
Steudel, E. G. 1853–1855. Synopsis Plantarum Glumacearum, Part 1. Synopsis Plantarum Graminearum. J. B. Metzler, Stuttgart.
Tateoka, T. 1958. Notes on some grasses, VIII. On leaf structure of *Arundinella* and *Garnotia*. Bot. Gaz. (Tokyo) 120: 101–109.
———. 1963. Notes on some grasses XV. Affinities and species relationship of *Arthropogon* and relatives, with reference to their leaf-structure. Bot. Mag. (Tokyo) 76: 286–291.
Watson, L. & M. J. Dallwitz. 1992. The Grass Genera of the World. C. A. B. International, Wallingford.
——— & M. J. Dallwitz. 1992 onward. Grass Genera of the World: Descriptions, Illustrations, Identification, and Information Retrieval; Including Synonyms, Morphology, Anatomy, Physiology, Phytochemistry, Cytology, Classification, Pathogens, World and Local Distribution, and References. Version: 28th May 1999. http://biodiversity.uno.edu/delta/
———, H. T. Clifford & M. J. Dallwitz. 1985. The classification of Poaceae: Subfamilies and supertribes. Austral. J. Bot. 33: 433–484.
Webster, R. D. 1988. Genera of the North American Paniceae (Poaceae: Panicoideae). Syst. Bot. 13: 576–609.
———. 1992. Character significance and generic similarities in the Paniceae (Poaceae: Panicoideae). Sida 15: 185–213.
———, J. H. Kirkbride & J. Valdés Reyna. 1989. New World genera of the Paniceae (Poaceae: Panicoideae). Sida 13: 393–417.
Wills, K. E., R. D. B. Whalley & J. J. Bruhl. 2000. Systematics studies in Paniceae (Poaceae): *Homopholis* and *Whalleya*, gen. et sp. nov. Austral. Syst. Bot. 13: 437–468.
Zuloaga, F. O. & T. R. Soderstrom. 1985. Classification of the outlying species of New World *Panicum* (Poaceae: Paniceae). Smithsonian Contr. Bot. 59: 1–63.
———, R. P. Ellis & O. Morrone. 1992. A revision of *Panicum* subgenus *Phanopyrum* section *Laxa* (Poaceae: Panicoideae: Paniceae). Ann. Missouri Bot. Gard. 79: 770–818.
———, O. Morrone & L. Giussani. 2000. A cladistic analysis of the Paniceae: A preliminary approach. Pp. 123–135 *in* S. W. L. Jacobs & J. Everett (editors), Grasses: Systematics and Evolution. CSIRO, Melbourne.

Appendix 1.

Morphological characters

Inflorescence: character 1

Inflorescence morphology is variable in the positioning of the primary branches. In this study we designate as compact those inflorescences in which the primary branches form an angle of less than 30° from the main rachis; and as open those inflorescences with the primary branches more than 30° (usually 45°).

Pilose callus: character 2; *stipe:* character 4; and *base torose:* character 33

The presence of a differentiated structure at the base of spikelet has been termed a callus or stipe (Burkart, 1969; Rosengurtt et al., 1970; Nicora & Rúgolo de Agrasar, 1987; Judziewicz, 1990; Watson & Dallwitz, 1992; Renvoize, 1998). Webster (1992) mentioned the presence of a callus in 21 genera of Paniceae, but did not distinguish whether the structures were homologous. Since such general designations may involve very different structures or a combination of different structures, they must be more precisely defined in order to estimate homology.

In this study we define a pilose callus to be a whorl of unicellular macrohairs at the base of the spikelet that is clearly differentiated from the rest of the surface. A stipe is defined as a sharply pointed structure derived from the fusion of the base of the lower glume with the enlarged rachilla internode between the lower and upper glume. This occurs in *Arthropogon piptostachyus*. A toroid base is defined as an enlarged ringlike growth formed from the lateral enlargement of the rachilla of the internode between the lower glume and the upper floret. In *Streptostachys* this structure functions as an elaiosome at spikelet maturity (Morrone & Zuloaga, 1991).

Spikelet compression: character 3

The compression of the spikelets is a diagnostic character for distinguishing genera of the Paniceae (Clayton & Renvoize, 1986; Webster, 1988). Lateral compression is recognized by the form of the bracts, which are usually keeled and folded along the length of the midnerve. Dorsal compression is recognized by the form of the bracts, which are rounded or flattened dorsally and with the plane of compression opposite to the plane formed by the midnerve of the spikelet bracts.

Spikelet bracts: characters 5, 6, 7, 8, 9, 10, 13, 14, 15, 22, 26, 28, 29, 31, and 34

Genera of Paniceae usually possess two-flowered spikelets with two glumes, one lower lemma and palea forming the lower floret and one upper lemma and palea forming the upper floret. However, variations of this basic organizational plan occur in many of the genera in our study. *Thyridolepis, Homolepis, Streptostachys, Digitaria, Homo-*

pholis, Melinis, Achlaena, and *Arthropogon* s.l. all possess the lower and upper glume, the lower lemma, and the upper lemma and palea. The lower palea is present in *Homolepis, Anthaenantia, Arthropogon villosus, A. filifolius, A. xerachne,* and *A. lanceolatus,* but is variously reduced in *Thyridolepis, Streptostachys, Digitaria, Melinis,* and *Arthropogon bolivianus.*

Another reduction series affects the lower glume. The presence or absence of the lower glume is an important character for recognizing genera in the Panicoideae (Webster, 1988; Judziewicz, 1990; Renvoize, 1998). Clayton and Renvoize (1986) and Watson and Dallwitz (1992) treated *Anthaenantia* and *Leptocoryphium* as related to each other and characterized both by the absence of a lower glume. A third reduction series occurs in *Reynaudia* in which both the lower and upper palea are absent.

Texture of lower glume: character 34

The texture of the lower glume in the Paniceae varies from herbaceous, hyaline, transparent, or rigidly setaceous and awnlike.

Texture of upper glume and lower lemma: characters 28 and 29

The texture of the upper glume and lower lemma in Paniceae is variable; it may be coriaceous or membranous. The apex of the upper glume and lower lemma is entire or bilobed; the midnerve may be prolonged into a slender awn. When developed, the awn may be straight or twisted and in the latter case is then hygroscopic. The nerves of the upper glume and lower lemma may be delicate or enlarged, in the last case with deep intercostal grooves.

Upper floret: characters 12, 16, and 26

The most diagnostic characters in the Paniceae involve the upper lemma and upper palea, which together form the upper floret (Chase, 1911; Webster, 1988). Similar to the spikelets as a whole, the upper floret may be laterally or dorsally compressed. Lemma texture is an important character in the delimitation of genera of Paniceae (Chase, 1911; Blake, 1958; Hsu, 1965; Clayton & Renvoize, 1986). The lemma of the taxa in this study may be coriaceous or hyaline, and its color varies from straw-colored to castaneous or translucent. Since the texture and color vary with the development of the spikelets, care was taken to only use only mature spikelets, and when possible, one enclosing a mature caryopsis. In the absence of a hypothesis about the evolution of the color of the upper floret, this character was treated as an unordered one.

Hilum: character 25

Hilum types in the Paniceae vary considerably, but there are no general studies dealing with evolution of this character in this tribe. In the Paniceae the punctate type of hilum is most frequent, followed by the linear type. Zuloaga and Soderstrom (1985), Filgueiras (1986), Sendulsky et al. (1987), and Morrone and Zuloaga (1991) considered the hilum type to be a useful taxonomic character. For this study, the hilum was considered to be linear when it occupies the entire length of the caryopsis and punctate when it is no longer than ¼ as long as the caryopsis.

Germination lid: character 27

The presence of a well-defined germination lid is variable in the Paniceae. Blake (1958) indicated that its presence constituted a significant character in the Paniceae, but it has been underutilized.

ANATOMICAL CHARACTERS (CHARACTERS 18–21, 23, 30)

Characters 19 and 20 deal with the photosynthetic pathway and with the Kranz syndrome. Brown (1977) and Ellis (1977, 1988) established the importance of anatomical leaf characters for analyzing relationships within the Paniceae. These authors demonstrated the presence of diverse anatomical and physiological types in the tribe, including C_3 and C_4 types in the plants under investigation, with the latter further subdivided as subtypes nicotinamide adenine dinucleotide co-factor malic enzyme (NAD-me), phosphoenol pyruvate carboxykinase (PCK), k and nicotinamide adenine dinucleotide phosphate co-factor malic enzyme (NADP-me). In order to establish the homologies of the different structures involved in each anatomical type, we follow the criteria proposed by Brown (1977) and Dengler et al. (1985). They considered that the Kranz sheath of the NADP-me anatomical type is homologous to the mestome (= MS) sheath of C_3 plants, and that the Kranz sheath of the NAD-me or PEP-ck is homologous to the parenchyma (= PS) sheath of C_3 grasses.

The distinctive Kranz cells (character 20) are distributed in the mesophyll and are not associated directly with vascular tissue. As Tateoka (1958) had done earlier, Hattersley and Watson (1992) interpreted distinctive Kranz cells to be vestiges or extreme reductions of the sheaths of minor vascular bundles. However, the absence of associated vascular tissue with the distinctive Kranz cells and the lack of ontogenetic studies to interpret homology in relation to meristematic origin caused us to treat this character separately.

The presence of fusoid cells (character 21) is diagnostic for the Bambusoideae (Metcalfe, 1960; Jacques-Félix, 1962; Soderstrom & Ellis, 1987; Ellis, 1987), but they also occur in *Homolepis* (Watson et al., 1985; Renvoize, 1987; Watson & Dallwitz, 1992), *Streptostachys* (Clayton & Renvoize, 1986; Renvoize, 1987), and in species of *Panicum* sect. *Laxa* (Killeen & Clark, 1986; Zuloaga et al., 1992). In these panicoid taxa the fusoid cells are globose, transversally enlarged, with thin cell walls and without intercellular spaces evident in transverse sections, whereas intercellular spaces occur in the Bambusoideae.

APPENDIX 2. LIST OF SPECIMENS EXAMINED FOR THE CLADISTIC ANALYSIS

Achlaena piptostachya Griseb.

CUBA: Without locality, *Wright 3487* (isotypes, US-927103, fragment, US-75310); hills SE of Soledad, 30 Nov. 1926, *Hitchcock 23333* (US). **Isla de la Juventud:** San Pedro region, white-sand sabanas, 18 Mar. 1954, *Killip 43960* (US); near Nueva Gerona, 17 Dec. 1903, *Curtiss 2 '6* (US). **Pinar del Río:** along camino Aguacate from B thía Honda to Baños Aguacate, 11 Dec. 1910, *Wilson 9 15* (US); Taco-Taco, Sierra de los Organos, 400–500 m, 1 Nov. 1941, *Morton 4365* (US).

A toparadisium chapadense Filg. et al.

BRAZIL. **Goiás:** Chapada dos Veadeiros, município of A to Paraíso, ca. 26 km a Oeste of Alto Paraíso, ca. 14° 0 'S–47°30'W, campo rupestre, estrada para Colinas do S l Goiás, 5 Sep. 1994, *T. S. Filgueiras & M. L. Fonseca 2 87* (isotype, SI).

A toparadisium scabrum (Pilg. & Kuhlm.) Filg. et al. var. ***scabrum***

BRAZIL. **Mato Grosso:** Cáceres, Agua Quente, *Kuhlm nn 1853* (isotype, US-A0865392).

A toparadisium scabrum (Pilg. & Kuhlm.) Filg. et al. var. ***bolivianum*** (Filg.) Filg. et al.

BOLIVIA. **Santa Cruz:** near the Arches, 5 km N of S ntiago of Chiquitos, 700 m, 2 Sep. 1942, *Cutler 7023* (h lotype, US-2013303).

Altoparadisium scabrum (Pilg. & Kuhlm.) Filg. et al. var. ***rupestre*** (Filg.) Filg. et al.

BRAZIL. **Goiás:** Serra do Caiapó, 17°12′S, 51°47′W, ca. 50 km S of Caiapônia on road to Jataí, 26 Oct. 1964, *Irwin & Soderstrom 7390* (fragment of isotype, US).

Anthaenantia rufa (Elliot) Schult.

U.S.A. **Texas:** 5 mi. NW of Groveton, *Gould 12001* (SI).

Anthaenantia villosa (Michx.) P. Beauv.

U.S.A. **Florida:** ca. 15 mi. N of Pensacola, *Silveus 5622* (US).

Arthropogon filifolius Filg.

BRAZIL. **Distrito Federal:** Cabeça do Veado, Capim de espiguetas aristadas, crescendo em brejo recém queimado, 15 Sep. 1980, *T. S. Filgueiras 671* (US-2946634). **Minas Gerais:** Uberlandia, just N of town, left side RR, 850–870 m, 15 Mar. 1930, *Chase 11261 1/2* (US).

Arthropogon lanceolatus Filg.

BRAZIL. **Minas Gerais:** Furnas, Ribeirão das Pacas-Cascatas, 28 July 1966, *Emygdio 2308* (isotype, US-2979541).

Arthropogon villosus Nees

BRAZIL. "Habitat in campis altis Chapada of Paranan provinciae Minarum versus Goyaz, et ad Rio Fermoso," *Martius s.n.* (isotype fragment, US-865393). **Bahia:** 22 km S of the Rio Galheirao along Hwy. BR-020, 860 m, 8 Apr. 1976, *Davidse et al. 12222* (US). **Mato Grosso:** Braco, Rio Arinos, 26 Sep. 1943, *Baldwin 3041* (US). **Minas Gerais:** Carmo, 26 Sep. 1948, *Macedo 1246* (US); S. Vicente, 10 Nov. 1956, *Macedo 2711* (US). **São Paulo:** Serra da Cunha, 1200 m, 14 Mar. 1939, *Kuhlman & Gehrt s.n.* (US-2917179); Mun. S. José dos Campos, 6.8–7.2 km ao SSE em linha reta de praça principal de São José dos Campos, 12 Sep. 1962, *Mimura 557* (US).

Arthropogon xerachne Ekman

BRAZIL. **São Paulo:** S. José dos Campos, in campo, 1910, *Löfgren 243* (syntype, US-3168439, fragment); Mun. S. José dos Campos, 6.8–7.2 km ao SSE em linha reta da praça principal of São José dos Campos, 12 Sep. 1962, *Mimura 563* (US); Campinas, 12 Dec. 1940, *Viegas & Lima s.n.* (US-1764757). **Paraná:** without locality, 23 June 1910, *Dusén 10002* (syntype, US-3168438, fragment); Jaguariahyva, in campo cerrado, 730 m, 30 Nov. 1915, *Dusén 17367* (SI), 22 Oct. 1911, *Dusén 13231* (SI, US); São José dos Campos, Sep. 1908, *Löfgren 3820* (US); Mun. S. José dos Campos, 7.5 km S of São José dos Campos, along highway to Paraibuna, 31 May 1961, *Eiten & Sendulsky 2867* (US).

Digitaria ciliaris (Retz.) Koel.

ARGENTINA. **Santiago del Estero:** Sierra de Guasayán, Quebrada de Conzo, 16 Feb. 1989, *Ulibarri 1566* (SI).

Digitaria sanguinalis (L.) Scop.

U.S.A. **Florida:** Altamonte Spring, 25 Apr. 1959, *Schallert 10441* (SI).

Digitaria setigera Roth

COLOMBIA. **Antioquia:** Mun. Andes, 12.5 km de los Andes hacia el Corregimiento de Tapartó, 24 Aug. 1988, *Fonnegra et al. 2476* (SI).

Homolepis aturensis (Kunth) Chase

BOLIVIA. **Beni:** Guayamerim, *Krapovickas & Schinini 35109* (CTES). BRAZIL. **Amazonas:** Barcelãos Negro river, *Baldwin 3330* (US). **Pernambuco:** Recife, *Pickel 3608* (US). COLOMBIA. **Cauca:** Chisquío, Finca de Derrumbes, *Asplund 10558* (US).

Homopholis belsonii C. E. Hubb.

AUSTRALIA. Jondaryan, Darling Downs District, 1250 ft., 12 Feb. 1938, *Blake 13252* (MO).

Leptocoryphium lanatum (Kunth) Nees

ARGENTINA. **Corrientes:** Dpto. Capital, 15 km al S de la ciudad de Corrientes, Riachuelo, 16 Dec. 1983, *Hunziker et al. 11091* (SI). **Entre Ríos:** Depto. Colón, Palmar, a 13 km E de Ruta Nac. 14, camino al Palmar, 17 Jan. 1976, *Romanczuk et al. 56* (SI). **Misiones:** Dpto. San Ignacio, San Ignacio, 11 Oct. 1975, *Zuloaga & Deginani 450* (SI). BOLIVIA. **Santa Cruz:** Buena Vista, 30 Sep. 1916, *Steinbach 2882* (SI). PARAGUAY. **Itapúa:** Isla Yaciretá, camino al aeródromo de Yaciretá, 27°22′S, 56°38′W, 23 Apr. 1992, *Morrone & Pensiero 237* (SI).

Melinis minutiflora P. Beauv.

BRAZIL. **Bahia:** Mun. de Ilhéus, estrada Olivenca-Maruim, entre los kms 7–10, restinga arbórea, 19 May 1985, *Zuloaga et al. 2478* (SI). **Paraná:** Porto Amazonas, ad ripam fluminis Rio Iguassú, 800 m, 10 June 1914, *Dusén 15154* (SI). **Santa Catarina:** Sombrio, 50 m, 29 May 1945, *Reitz 1130* (SI). **Pernambuco:** Tapera, Sep. 1930, *Pickel 97* (SI). PERU. **Cajamarca:** Prov. Cutervo, a 22 km sobre la carretera Cutervo–Sócota, 2450 m, 15 June 1980, *Sánchez Vega 2296* (SI). VENEZUELA. **Miranda:** Los Teques, faldas de cerros, 14 Mar. 1946, *Burkart 16012* (SI).

Reynaudia filiformis (Spreng. ex Schult.) Kunth

DOMINICAN REPUBLIC. Llano Costero, Higüey, savanna 2 km W of town, 17 Apr. 1929, *Ekman 12208* (MO).

Streptostachys asperifolia Desv.

BRAZIL. **Amapá:** Porto Platón, *Mattos 10120* (SP, US). **Goiás:** 2 km N of Araguainia, *Irwin et al. 21133* (F, NY, US). **Pará:** near Soure, Marajó Island, *Goeldi 299* (US). VENEZUELA. **Sucre:** 7 km E of the Mochima Hwy., intersection along Hwy. 9 between Cumaná and Puerto la Cruz, *Davidse 5044* (MO).

Thyridolepis multiculmis (Pilg.) S. T. Blake

AUSTRALIA. **Northern Territory:** Uluru National Park, on the Docker River road near the W park boundary, 55 km WNW of the Ranger Station, 25°17′S, 130°40′W, 18 Aug. 1988, *Lazarides & Palmer 535* (MO).

Volume 88, Number 2, pp. 163–372 of the ANNALS OF THE MISSOURI BOTANICAL GARDEN
was published on June 29, 2001.

Missouri Botanical Garden Library
3 1753 00299 5824

ANNALS OF THE MISSOURI BOTANICAL GARDEN
AND MISSOURI BOTANICAL GARDEN ANNUAL REPORT
ARE NOW AVAILABLE IN JSTOR!

JSTOR®, a not-for-profit organization, is an important endeavor dedicated to helping the scholarly community take advantage of advances in electronic technologies. The JSTOR Archive makes available the complete backfiles of *Annals of The Missouri Botanical Garden*, *Missouri Botanical Garden Annual Report (1890-1912)*, and more than 100 other scholarly journals to researchers through participating libraries.

JSTOR has recently announced the release of its Ecology & Botany Collection. Developed in cooperation with the Ecological Society of America, the Collection includes the archives of twenty-nine journals, offering online access to more than 1 million pages of important historic literature.

The Ecology & Botany Collection

American Journal of Botany
American Midland Naturalist
American Naturalist
Annals of the Missouri Botanical Garden
Annual Review of Ecology and Systematics
Biotropica
Brittonia
Conservation Biology
Diversity and Distributions
Ecological Applications
Ecological Monographs
Ecology
Evolution
Functional Ecology
Global Ecology and Biogeography Letters
International Journal of Plant Sciences
Journal of Animal Ecology
Journal of Applied Ecology
Journal of Biogeography
Journal of Ecology
Journal of Tropical Ecology
Journal of The Torrey Botanical Society
Limnology and Oceanography
Missouri Botanical Garden Annual Report
New Phytologist
Paleobiology
Quarterly Review of Biology
Systematic Biology
Systematic Botany

Information regarding JSTOR is available at http://www.jstor.org
120 Fifth Avenue, New York, NY 10011

CONTENTS

Phylogenetic Analysis of Asterids Based on Sequences of Four Genes ——— *Dirk C. Albach, Pamela S. Soltis, Douglas E. Soltis & Richard G. Olmstead* 163

The Phylogenetic Status of *Polyscias* (Araliaceae) Based on Nuclear ITS Sequence Data ——— *Gregory M. Plunkett, Porter P. Lowry II & Michele K. Burke* 213

Evolution, Phylogeny, and Systematics of the Juglandaceae ——— *Paul S. Manos & Donald E. Stone* 231

The Utility of Morphological Characters for Inferring Phylogeny in Scandiceae Subtribe Scandicinae (Apiaceae) ——— *Krzysztof Spalik & Stephen R. Downie* 270

The Genus *Hypoxis* (Hypoxidaceae) in Central Africa ——— *Justyna Wiland-Szymańska* 302

The Establishment of the New Genus *Altoparadisium* and a Reevaluation of *Arthropogon* (Poaceae, Paniceae) ——— *Tarciso S. Filgueiras, Gerrit Davidse, Fernando O. Zuloaga & Osvaldo Morrone* 351

Cover illustration. Top: *Pronuba yuccasella*, female, in the act of gathering pollen from the anthers of *Yucca filamentosa*. Bottom: Pistils of *Y. filamentosa*, showing, from left, a deformed specimen and varying lengths of the stamens. From *Annual Rep. Missouri Bot. Gard.* 1892, The Yucca Moth and Yucca Pollination, by C. V. Riley. See Coevolution, the 46th Annual Systematics Symposium of the Missouri Botanical Garden: Introduction, first issue of this volume.

QK1
.A47573
C.1

Annals of the Missouri Botanical Garden

2001

Volume 88
Number 3

Volume 88, Number 3
Summer 2001

Annals of the
Missouri Botanical Garden

The Annals, published quarterly, contains papers, primarily in systematic botany, contributed from the Missouri Botanical Garden, St. Louis. Papers originating outside the Garden will also be accepted. All manuscripts are peer-reviewed by qualified, independent reviewers. Authors should write the Managing Editor for information concerning arrangements for publishing in the ANNALS. Instructions to Authors are printed in the back of the last issue of each volume and are also available online at www.mobot.org/mbgpress.

Editorial Committee

Victoria C. Hollowell
Editor,
Missouri Botanical Garden

Amy Scheuler McPherson
Managing Editor,
Missouri Botanical Garden

Diana Gunter
Associate Editor,
Missouri Botanical Garden

Aida Kadunic
Senior Secretary

Barbara Mack
Administrative Assistant

Ihsan A. Al-Shehbaz
Missouri Botanical Garden

Gerrit Davidse
Missouri Botanical Garden

Roy E. Gereau
Missouri Botanical Garden

Peter Goldblatt
Missouri Botanical Garden

Gordon McPherson
Missouri Botanical Garden

P. Mick Richardson
Missouri Botanical Garden

Henk van der Werff
Missouri Botanical Garden

For subscription information contact ANNALS OF THE MISSOURI GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897. Subscription price for 2002 is $140 per volume U.S., $150 Canada & Mexico, $175 all other countries. Four issues per volume. The journal *Novon* is included in the subscription price of the ANNALS.

annals@mobot.org (editorial queries)
http://www.mobot.org/mbgpress

THE ANNALS OF THE MISSOURI BOTANICAL GARDEN (ISSN 0026-6493) is published quarterly by the Missouri Botanical Garden, 2345 Tower Grove Avenue, St. Louis, MO 63110. Periodicals postage paid at St. Louis, MO and additional mailing offices. POSTMASTER: Send address changes to ANNALS OF THE MISSOURI BOTANICAL GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897.

© Missouri Botanical Garden 2001

The mission of the Missouri Botanical Garden is to discover and share knowledge about plants and their environment, in order to preserve and enrich life.

This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

Volume 88
Number 3
2001

Annals
of the
Missouri
Botanical
Garden

PHYLOGENY AND SUBFAMILIAL CLASSIFICATION OF THE GRASSES (POACEAE)[1]

Grass Phylogeny Working Group[2,3]

ABSTRACT

A large collaborative effort has yielded a comprehensive study of the phylogeny and a new subfamilial classification of the grass family (Poaceae/Gramineae). The study was conducted on an integrated and representative set of 62 grasses (0.6% of the species and ca. 8% of the genera) plus four outgroup taxa using six molecular sequence data sets (*ndh*F, *rbc*L, *rpo*C2, *phy*B, ITS2, and GBSSI or *waxy*), chloroplast restriction site data, and morphological data. A parsimony analysis using 2143 informative characters (the combined analysis) resulted in a single most parsimonious tree of 8752 steps with an RI of 0.556 and bootstrap support of > 90% for more than half of the internal nodes. Significant relationships that appear consistently in all analyses of all data sets and are strongly supported by the combined analysis include the following: Joinvilleaceae are sister to a monophyletic Poaceae; the earliest diverging lineages of the Poaceae are Anomochlooideae, Pharoideae, and Puelioideae, respectively; and all remaining grasses form a clade. Multiple monophyletic clades were recovered, including Bambusoideae s. str., Ehrhartoideae, Pooideae s.l., Aristidoideae, Danthonioideae, Chloridoideae s. str., Chloridoideae s.l., Panicoideae, Parianeae, Olyreae s. str., Oryzeae, Stipeae, Meliceae, *Lygeum* + *Nardus*, and *Molinia* + *Phragmites*. The PACCAD Clade is monophyletic, containing Aristidoideae, Danthonioideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Panicoideae, *Eriachne*, *Micraira*, and *Gynerium*. Based on the phylogeny, a classification of 11 previously published subfamilies (Anomochlooideae, Pharoideae, Puelioideae, Bambusoideae, Ehrhartoideae, Pooideae, Aristidoideae, Arundinoideae, Chloridoideae, Centothecoideae, and Panicoideae) and 1 new subfamily (Danthonioideae) is proposed. Several changes in the circumscription of traditionally recognized subfamilies are included. Previous phylogenetic work and classifications are reviewed in relation to this classification and circumscription, and major characteristics of each subfamily are discussed and described. The matrix, trees, and updated data matrix are available at ⟨http://www.virtualherbarium.org/grass/gpwg/default.htm⟩.

Key words: cereals, classification, DNA sequence data, evolution, grass, phylogeny, Poaceae.

[1] Work presented here was supported in part by NSF grants DEB-9806584 and DEB-9806877 to LGC, DEB-9727000 to JID, DEB-9419748 and DEB-9815392 to EAK, and BIR-9508467 to SYM. Miwa Kojima prepared the line illustrations of leaf anatomy and spikelets. We thank T. Cope, J. Everett, S. W. L. Jacobs, S. Phillips, S. A. Renvoize, and P. F. Stevens for helpful comments on the manuscript.

[2] This paper is to be cited as authored by the Grass Phylogeny Working Group, or GPWG. The group includes the following members, listed here in alphabetical order; there is no senior author. Nigel P. Barker, Department of Botany, Rhodes University, P.O. Box 94, Grahamstown, 6140, South Africa; Lynn G. Clark, Department of Botany, Iowa State University, Ames, Iowa 50011-1020, U.S.A.; Jerrold I. Davis, L. H. Bailey Hortorium, Cornell University, 462 Mann Library, Ithaca, New York 14853, U.S.A.; Melvin R. Duvall, Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115-2861, U.S.A.; Gerald F. Guala, Fairchild Tropical Garden, 11935 Old Cutler Road, Miami, Florida 33156, U.S.A.; Catherine Hsiao, 6005 Crossmont Court, San Jose, California 95120, U.S.A.; Elizabeth A. Kellogg, Department of Biology, University of Missouri-St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121, U.S.A.; H. Peter Linder, Institut für Systematische Botanik, Zollikerstrasse 107, CH-8008, Zurich, Switzerland;

The economic and ecological significance of the grasses (Poaceae) has led to widespread interest in their evolution and classification. The cereals, sugarcane, bamboos, and forage and weedy grasses are of pre-eminent importance in human economy. Grasses, which occur in virtually every terrestrial habitat, cover as much as one-fifth of the Earth's land surface (Shantz, 1954). Long recognized as a "natural" group, the grass family includes approximately 10,000 species in over 700 genera (Dahlgren et al., 1985; Tzvelev, 1989; Watson & Dallwitz, 1992; Renvoize & Clayton, 1992). Efforts to produce a comprehensive, formal taxonomic structure of the family began over 200 years ago, while serious study of grass evolution began late in the 19th century.

The Grass Phylogeny Working Group (GPWG) was established in 1996 to (1) combine a series of existing data sets to produce a comprehensive phylogeny for the grass family; (2) focus taxon sampling in the development of existing and future data sets; and (3) reevaluate the subfamilial classification of the grass family based on the results of the phylogenetic analyses. We combined and performed cladistic analyses on eight data sets (one structural, four plastome, and three nuclear) from 62 grasses and 1 outgroup taxa. The phylogenetic results and a revised subfamilial classification of the grass family are presented in this paper.

Review of Grass Phylogeny and Classification

Historically, the Poaceae were thought to be related to Cyperaceae (Engler, 1892; Cronquist, 1981) based on floral reduction and chemical characters, but evidence accumulated during the past 15 years unequivocally shows that the similarities are convergent. Phylogenetic studies based on morphological and molecular characters show that the grasses are most closely related to Joinvilleaceae, Restionaceae, Anarthriaceae, and Ecdeiocoleaceae, and are somewhat more distantly related to Flagellariaceae (Dahlgren et al., 1985; Campbell & Kellogg, 1987; Linder, 1987; Doyle et al., 1992; Kellogg & Linder, 1995; Briggs et al., 2000); this group forms the graminoid clade, a subset of the order Poales (APG, 1998). A sister-group relationship between Poaceae and Joinvilleaceae consistently has been supported (Campbell & Kellogg, 1987; Doyle et al., 1992; Clark et al., 1995; Soreng & Davis, 1998), although *rbc*L sequence data suggest that Joinvilleaceae + Ecdeiocoleaceae is the sister clade to Poaceae (Briggs et al., 2000).

The grass family was recognized as distinctive and coherent long before the term monophyly was ever applied. The fruit (caryopsis) is unique to the family, with the outer integument developmentally fused to the inner wall of the ovary. The embryo is lateral and, unlike most monocot embryos, is highly differentiated, with clear shoot and root meristems, leaves and vascular system. The pollen, as is typical for the whole order Poales, has only one aperture, but in grasses the pollen wall lacks scrobiculi. In all but the earliest-diverging lineage, the grass spikelet consists of a set of distichous bracts, the basal two empty (glumes) with a series of one to many bracts (lemmas) above, each lemma subtending a contracted floral axis on which is borne a presumed prophyll (palea), two or three reduced perianth parts (lodicules), the androecium, and the gynoecium (see discussion under Spikelet).

Although subdivision of the grasses into groups we today call tribes began in the 18th century (see reviews in Calderón & Soderstrom, 1980; Gould & Shaw, 1983; Pohl, 1987), works by Brown (1810, 1814) represent the earliest attempt to define groups of tribes, or what we now call subfamilies. Brown (1814) divided the grasses into the tribe Paniceae (roughly equivalent to the modern Panicoideae) and the tribe Poaceae (roughly equivalent to the Festucoideae of Hitchcock & Chase, 1950) based on spikelet compression, articulation, and floret number. Brown is credited with describing grass spikelets in detail and recognizing them as branched structures, as well as noting the tendency for the panicoids to grow in warm climates and the pooids in cooler climates (Gould & Shaw, 1983; Pohl, 1987). Brown's division of the family into two major groups was formalized by Bentham (1878), was retained by Bentham and Hooker (1883) and Hackel (1887), and persisted well into the 20th century (e.g., Hitchcock, 1935; Hitchcock & Chase, 1950).

Several classifications for the grasses based on spikelet and inflorescence morphology were pro-

Roberta J. Mason-Gamer, Department of Biological Sciences, University of Idaho, Moscow, Idaho 83844, U.S.A.; Sarah Y. Mathews, Division of Biological Sciences, University of Missouri-Columbia, 226 Tucker Hall, Columbia, Missouri 56211, U.S.A.; Mark P. Simmons, The Ohio State University Herbarium, Ohio State University, 1315 Kinnear Road, Columbus, Ohio 43212, U.S.A.; Robert J. Soreng, Department of Botany, Natural History Museum, Smithsonian Institution, Washington, D.C. 20560-0166, U.S.A.; Russell E. Spangler, Department of Ecology, Evolution, and Behavior, University of Minnesota, 1987 Upper Buford Circle, St. Paul, Minnesota 55108, U.S.A.

[3] Author for correspondence: Elizabeth A. Kellogg, tkellogg@umsl.edu.

posed in the 19th century (see reviews in Calderón & Soderstrom, 1980; Gould & Shaw, 1983; Campbell, 1985; Pohl, 1987), with usually nine or ten tribes recognized. Some tribes, for example Paniceae, Andropogoneae, and Bambuseae, contain largely the same genera now as nearly 200 years ago. Others, such as the various "pooid" tribes, included disparate elements and are now seen as artificial.

Whether explicit or not, a different perspective on the evolution of grasses and relationships within the family began to emerge by the end of the 19th century. Workers such as Čelakovský (1889), Goebel (1895), and Schuster (1910) carefully analyzed spikelet structure and proposed that *Streptochaeta*, or something very much like it, was representative of the most primitive grasses. With the development of leaf anatomical (Duval-Jouve, 1875; Prat, 1932), embryological (van Tieghem, 1897), and cytological (Avdulov, 1931) data, a profound reassessment of evolutionary relationships among grasses began. Additional data on embryo anatomy (Reeder, 1957, 1961, 1962), starch grains (Tateoka, 1962), lodicules (Jirásek & Jozífová, 1968; Guédès & Dupuy, 1976), and leaf anatomy (Brown, 1958; Metcalfe, 1960) accumulated and were also incorporated into evolutionary and classification schemes. Several classification systems were published in the 20th century (e.g., Roshevits, 1937, 1946; Tateoka, 1957; Prat, 1960; Stebbins & Crampton, 1961; Jacques-Félix, 1962; Caro, 1982; Clayton & Renvoize, 1986; Tzvelev, 1989; Renvoize & Clayton, 1992; Watson & Dallwitz, 1992); major ones that are global in scope are compared in Table 1. The number of subfamilies recognized ranged from 2 (Tzvelev, 1989) to 13 (Caro, 1982). All but the Watson and Dallwitz (1992) classification, which is avowedly phenetic, were based on presumed evolutionary relationships. The major change was the subdivision of the old Festucoideae (or Pooideae) into several subfamilies; Panicoideae were retained almost without modification. Other differences among the major classification systems primarily relate to the treatment of Arundinoideae and Bambusoideae. Clayton and Renvoize (1986) in particular published a number of diagrams depicting relationships based on their synthesis of knowledge at that time. These diagrams have served as a starting point for much subsequent work.

Phenetic analyses of the grass family generally found groups consistent with the five or six subfamilies commonly recognized by the mid 1980s. Hilu and Wright (1982), in a cluster analysis of morphological and anatomical data, found eight major groups with strong support. Except for the cluster of *Diarrhena*, *Nardus*, and *Lygeum*, the remaining seven clusters corresponded to the subfamilies Festucoideae, Oryzoideae, Arundinoideae, Centothecoideae, Panicoideae, Eragrostoideae, and Bambusoideae. Watson et al. (1985) used the DELTA system to conduct comprehensive phenetic analyses of the family, and their character list continues to be developed. Watson and Dallwitz (1992) initially recognized five subfamilies and subsequently updated their classification to include seven (Watson & Dallwitz, 1999; http://biodiversity.uno.edu/delta/grass/www/class.htm); these are Stipoideae, Pooideae, Bambusoideae, Centothecoideae, Arundinoideae, Chloridoideae, and Panicoideae. Subsequent phenetic analyses of immunological data (Esen & Hilu, 1989) and plastid DNA reassociation (Hilu & Johnson, 1991) were limited in sampling but in each case produced four major groups.

Only within the past 15 years have cladistic methods been applied to questions of grass phylogeny and evolution. The first attempt to produce an explicit hypothesis of relationships was the morphological phylogeny of Kellogg and Campbell (1987), who analyzed 33 characters scored for virtually all grass genera. The pooids (including Stipeae), Panicoideae, Chloridoideae, and Bambusoideae were consistently monophyletic in their analyses, but Arundinoideae were polyphyletic, and the pooid clade formed the basal lineage in the family. Bambusoideae s.l. (including herbaceous tribes such as Anomochloeae, Phareae, Streptochaeteae, and Streptogyneae) were interpreted as monophyletic based on the presence of arm and fusoid cells; several tribes often included in the traditional Bambusoideae were placed in other clades (e.g., Brachyelytreae, Diarrheneae, and Phaenospermatidae in the pooid clade).

Hamby and Zimmer (1988) and Doebley et al. (1990) published the first molecular phylogenies for the family, based respectively on ribosomal RNA and plastid gene *rbc*L (ribulose 1,5-bisphosphate carboxylase/oxygenase, large subunit) sequence data. Relatively few taxa were sampled in both studies, but both supported the core Pooideae as well as the group that came to be known as the PACC clade (Davis & Soreng, 1993), containing subfamilies Panicoideae, Arundinoideae, Centothecoideae, and Chloridoideae.

The first extensive application of molecular data to grass phylogeny was undertaken by Davis and Soreng (1993), using plastid DNA restriction site variation for 31 taxa representing the six subfamilies of Clayton and Renvoize (1986). This study marked the beginning of wider sampling in the traditional Bambusoideae (= Bambusoideae s.l.), long

Table 1. Comparison of the major 20th century classification systems of the Poaceae.

Roshevits (1946)	Tateoka (1957)	Prat (1960)	Caro (1982)	Clayton & Renvoize (1986)	Tzvelev (1989)	Watson & Dallwitz (1992)	Grass Phylogeny Working Group (present paper)
Bambusoideae	**Pharoideae**	**Bambusoideae**	**Bambusoideae**	**Bambusoideae**	**Bambusoideae**	**Bambusoideae**	**Bambusoideae**
Bambuseae	Bambuseae	Bambuseae s.l.	Bambuseae	Bambuseae	Bambuseae	Bambuseae	Bambuseae
"	"			"	Arundinarieae	"	"
"	"			"	Shibataeeae	"	"
"	"			"	Dendrocalameae	"	"
"	"			"	Melocanneae	"	"
"	"			"	Oxytenanthereae	"	"
		Oryzoideae	**Olyroideae**				
Olyreae	Olyreae	Olyreae	Olyreae	Olyreae	Olyreae	Olyreae	Olyreae
Parianeae	Parianeae	*Pariana*	?	Parianeae	Parianeae	"	"
In Olyreae	?		?	In Olyreae	Buergersiochloeae	"	"
			Anomochlooideae				**Anomochlooideae**
Anomochloeae	Anomochloeae	Incertae Sedis	Anomochloeae	Anomochloeae	Anomochloeae	Anomochloeae	Anomochloeae
			Streptochaetoideae				
Streptochaeteae	Streptochaeteae	*Streptochaeta*	Streptochaeteae	Streptochaeteae	Streptochaeteae	Streptochaeteae	Streptochaeteae
							Pharoideae
Phareae	?	In Olyreae	?	Phareae	Leptaspideae	Phareae	Phareae
							Puelioideae
In Bambuseae	?	In Bambuseae	?	In Bambuseae	Atractocarpeae	Puelieae	Puelieae
In Bambuseae	?	In Bambuseae	?	In Bambuseae	In Bambuseae	Guaduelleae	Guaduelleae
Pooideae							**Ehrhartoideae**
?	See Arundoideae	Incertae Sedis	?	Streptogyneae	Streptogyneae	Streptogyneae	Incertae Sedis
			Oryzoideae		**Pooideae**		
Oryzeae	Oryzeae	Oryzeae	Oryzeae	Oryzeae	Oryzeae	Oryzeae	Oryzeae
	?		?	Diarrheneae	Diarrheneae	Diarrheneae	See Pooideae
	See Arundoideae		?	Brachyelytreae	Brachyelytreae	Brachyelytreae	See Pooideae

Table 1. Continued.

Phenospermeae	See Arundoideae Phyllorachieae		? ?	Phaenospermatae Phyllorachideae	Phaenospermatae Phyllorachideae	Phaenospermatae Phyllorachideae	See Pooideae Phyllorachideae
			Ehrhartoideae				
In Oryzeae	See Arundoideae	Incertae Sedis	Ehrharteae	Ehrharteae	Ehrharteae	Ehrharteae	Ehrharteae
			Centhostecoideae	**Centothecoideae**			**Centothecoideae**
Centotheceae	See Arundoideae Micraireae	Incertae Sedis	Centhosteceae	Centotheceae	Centosteceae	Centotheceae	Centotheceae Thysanolaeneae
	Pooideae	**Festucoideae**	**Festucoideae**	**Pooideae**		**Pooideae**	**Pooideae**
Hordeae	Triticeae	Hordeae		Triticeae	Triticeae	Triticeae	Triticeae
Brachypodieae					Brachypodieae	Brachypodieae	Brachypodieae
Bromeae				Bromeae	Bromeae	Bromeae	Bromeae
Festuceae	Festuceae	Festuceae		Poeae	Poeae	Poeae	Poeae
Aveneae	Agrosteae	Aveneae		Aveneae	"	Aveneae	"
Agrostideae	"	Agrostideae		"	"	"	"
Phalarideae	"	Phalarideae		"	In Phleeae	"	"
				In Poeae	Ampelodesmeae	In Stipeae	Ampelodesmeae
					Phleeae	Seslerieae	In Poeae
Meliceae	See Arundoideae	In Festuceae		Meliceae	Meliceae	Meliceae	Meliceae
				Brylkinieae	Brylkinieae	"	Brylkinieae
	Monermeae	Monermeae		Hainardieae	?	In Poeae	In Poeae
Stipeae	See Arundoideae	Stipeae		Stipeae	Stipeae	See Arundinoideae	Stipeae
Nardeae	See Arundoideae	Incertae Sedis		Nardeae	Nardeae	See Arundinoideae	Nardeae
Lygeae	See Arundoideae	Incertae Sedis		Lygeae	Lygeeae	See Arundinoideae	Lygeeae
							Brachyelytreae
							Diarrheneae
							Phaenospermatidae
		Beckmannia					
	Arundoideae	**Phragmitiformes**	**Phragmitoideae**	**Arundinoideae**		**Arundinoideae**	**Arundinoideae**
	Stipeae					Stipeae	See Pooideae
						Steyermarkochloeae	See Panicoideae
	Nardeae					Nardeae	See Pooideae
	Lygeae					Lygeae	See Pooideae

Table 1. Continued.

Sporoboleae	In Chlorideae	Sporoboleae			"	"	"
				Leptureae	"	"	Leptureae
		various isolated genera					*Centropodia, Merxmuellera rangei*
	Panicoideae	**Panicoideae**	**Panicoideae**	**Panicoideae**		**Panicoideae**	**Panicoideae**
—	—	—	—	Steyermarkochloeae	Steyermarkochloeae	See Arundinoideae	Steyermarkochloeae
In Eragrosteae	?	?		Eriachneae	In Arundineae	See Arundinoideae	Incertae Sedis
—	?	?		Hubbardieae	?	In Isachneae	Hubbardieae
In Festuceae	Isachneae	Isachneae		Isachneae	Isachneae	Isachneae	Isachneae
Paniceae	Paniceae	Paniceae		Paniceae	Paniceae	Paniceae	Paniceae
Boivinelleae	Boivinelleae	Boivinelleae		"	"	"	"
Melinideae	Melinideae	Melinideae		"	"	"	"
In Paniceae	Anthephoreae	?		"	"	"	"
In Paniceae	Lecomtelleae	?		"	"	"	"
In Zoysieae	Trachyeae	?		"	"	"	"
In Zoysieae	?	?		"	"	Neurachneae	"
In Melinidae	?	Arthropogoneae		"	"	In Paniceae	"
Arundinelleae	See Arundoideae	See Phragmitiformes		Arundinelleae	Arundinelleae	Arundinelleae	Arundinelleae
Andropogoneae	Andropogoneae	Andropogoneae		Andropogoneae	Andropogoneae	Andropogoneae	Andropogoneae
Maydeae	Maydeae	Maydeae		"	"	Maydeae	"
		various isolated genera including *Phaenosperma*					
		Incertae Sedis					**Incertae Sedis**
		Anomochloa					Eriachneae
		Centotheca					Micraireae
		Ehrharta					Streptogyneae
		Lygeum, Nardus					*Cyperochloa*
		Micraira					*Gynerium*
		Streptogyna					
		Thysanolaena and other genera					

presumed to include the most ancestral elements of the grass family. Davis and Soreng's (1993) results supported an expanded pooid clade, the PACC (now PACCAD) clade, and suggested that the traditional Bambusoideae were not monophyletic.

Nadot et al. (1994) analyzed sequences of the plastid gene *rps*4 (ribosomal plastid small subunit, protein 4) for 26 genera of grasses. Their sampling was heavily weighted toward the pooid grasses, but they did include three genera of woody bamboos and *Zizania* and *Oryza* of the ehrhartoids (oryzoids). They recovered both a monophyletic pooid clade (including *Stipa*) and the PACC clade. The bambusoid/oryzoid taxa were paraphyletic and formed a polychotomy with the PACC clade. Cummings et al. (1994), using sequence data from the plastid *rpo*C2 (RNA polymerase II, β″ subunit) gene, sampled only 13 genera, but did derive a monophyletic PACC clade and a monophyletic pooid clade. The *rbc*L sequence analysis of Barker et al. (1995) focused on the subfamily Arundinoideae. Both the PACC and pooid clades were shown to be monophyletic, although the traditional Arundinoideae appeared as polyphyletic. Bambusoideae, represented by a woody bamboo (*Bambusa*) and Ehrhartoideae (*Zizania* and *Oryza*), were paraphyletic to the rest of the family.

Clark et al. (1995) were the first to include a broad sample of bambusoid and ehrhartoid taxa. Using *ndh*F (NADH dehydrogenase, subunit F) sequence data, they confirmed the polyphyly of the traditional Bambusoideae and demonstrated that *Anomochloa* and *Streptochaeta*, two broad-leaved Neotropical forest genera, formed the earliest diverging branch of the family, with *Pharus*, another broad-leaved tropical forest genus, constituting the next most basal branch. Their results also confirmed strong support for monophyly of the PACC clade, an expanded pooid clade (including Stipeae, Phaenospermatideae, Brachyelytreae, and Diarrheneae), a derived, monophyletic core bambusoid clade (Olyreae + Bambuseae), and the polyphyly of the traditional Arundinoideae. They also recovered a weakly supported clade including the core bambusoids, ehrhartoids, and pooids, which they named the BOP clade (here updated to the BEP Clade based on nomenclatural priority of Ehrhartoideae over Oryzoideae). They concluded that many features previously used to define the traditional Bambusoideae, including the presence of arm and fusoid cells and pseudopetiolate leaf blades among others, were probable synapomorphies for the family.

The *rbc*L study of Duvall and Morton (1996) confirmed the basal placement of *Anomochloa*, as well as the monophyly of the core Bambusoideae, in addition to supporting the PACC and pooid clades. The topology recovered by Liang and Hilu (1996) from analysis of *mat*K (maturase K) sequence data was similar to the *rbc*L topologies, with the PACC and pooid clades sister to each other, and *Oryza* sister to that clade and a woody bamboo sister to the whole family. By this time, reassessment of subfamilial classification was necessary; Clark and Judziewicz (1996) resurrected Anomochlooideae and Pharoideae to accommodate the basal lineages of the family, which could not be retained in a monophyletic Bambusoideae.

Soreng and Davis (1998) combined a structural data set (including morphological, anatomical, chromosomal, and biochemical characters as well as structural features of the chloroplast genome) and an expanded chloroplast restriction site data set to analyze phylogenetic relationships within the grass family. They confirmed the basal positions of Anomochlooideae and Pharoideae, monophyly of an expanded Pooideae, monophyly of Panicoideae, Centothecoideae, and Chloridoideae, and polyphyly of the traditional Arundinoideae. The core Bambusoideae, supported as monophyletic in other analyses along with the ehrhartoid grasses, appeared as a set of clades paraphyletic to the [*Brachyleytrum* + (Pooideae + PACC)] clade. Soreng and Davis (1998) also identified structural synapomorphies for major clades, including, for example, loss of the epiblast and gain of an elongated mesocotyl internode in the PACC clade.

Barker et al. (1999) used sequences of the grass-specific insert in the chloroplast gene *rpo*C2 (hereafter referred to only as *rpo*C2) to study relationships among a broad sample of "arundinoid" taxa. They were the first to include molecular data on such traditionally arundinoid genera as *Centropodia*, *Merxmuellera*, *Notochloe*, *Tribolium*, *Monachather*, *Pentaschistis*, *Prionanthium*, *Cortaderia*, and *Spartochloa*. Because Arundinoideae were known to be polyphyletic, previous classifications were not helpful in placing these genera. *rpo*C2 sequences of *Anomochloa* and *Streptochaeta* could not be aligned with those of other grasses, so their basal position could not be tested. Relationships among the Bambusoideae, Pooideae s.l., and the PACC clade varied depending on the analytical method and inclusion of phylogenetically informative insertion/deletion characters. Consistent with previous studies, they identified Panicoideae and Chloridoideae as monophyletic. They showed clearly that a large clade corresponding to Danthonioideae is monophyletic and that this corresponds at least in part to the clade with haustorial synergids identi-

fied by Verboom et al. (1994). They also showed that the genus *Merxmuellera* is polyphyletic, with one species, *M. rangei*, most closely related to *Centropodia* and the chloridoids.

Hilu et al. (1999) sequenced the chloroplast gene *mat*K for 62 species of Poaceae and produced a tree that was quite similar to those found in previous studies. *Streptochaeta* and *Anomochloa* were the earliest diverging lineages, although paraphyletic instead of monophyletic. The *mat*K data supported a PACC clade and a clade including Pooideae and Bambusoideae. Oryzoideae (= Ehrhartoideae) was sister to the PACC clade, rather than the pooid/bambusoid clade, but this was not strongly supported.

Zhang (2000) used the intron in the chloroplast gene *rpl*16 (ribosomal protein 16) to construct a phylogeny of the grasses and confirmed (again) the early divergence of *Anomochloa* and *Streptochaeta*, although his data did not support the monophyly of the pair. The next branch was *Pharus*. The *rpl*16 data supported a PACC clade and a BEP Clade; *Puelia olyriformis* was sister to the BEP Clade, with modest bootstrap support.

Analyses of nuclear sequence data have provided results complementary to those obtained for molecular plastid data sets. Mathews and Sharrock (1996) and Mathews et al. (2000) sequenced loci in the phytochrome gene family and resolved a topology similar to that derived from *ndh*F sequence data, although the phytochrome data provided significantly stronger support for the BEP Clade than did the *ndh*F data. Additionally, the basal positions of *Anomochloa*, *Pharus*, and *Puelia* (as noted in Zhang, 1996) were confirmed. Hsiao et al. (1999) inferred phylogenetic relationships within the grasses based on sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. As in the other studies, *Streptochaeta* and *Pharus* were resolved as the basal lineages, and monophyly of the PACC clade and monophyly of the Pooideae were strongly supported. Unlike previous studies, however, some of Hsiao et al.'s (1999) analyses found that the traditional Arundinoideae were monophyletic.

Combined analysis of sequence data from two chloroplast genes (*ndh*F, *rbc*L) and one nuclear gene (phytochrome B) provided strong support for the placement of *Puelia* + *Guaduella* as the next most basal lineage of the family after Anomochlooideae and Pharoideae (Clark et al., 2000). These results necessitated the description of a new subfamily, the Puelioideae.

Mapping studies of the nuclear genome are in their infancy, but genome rearrangements are consistent with certain broad phylogenetic groups (Kellogg, 1998). Unique combinations of linkage groups are synapomorphic for subfamilies Pooideae (Moore et al., 1995; Gale & Devos, 1998), Panicoideae (Moore et al., 1995; Gale & Devos, 1998; Wilson et al., 1999), and Ehrhartoideae (Kennard et al., 1999). In addition, unique linkages support monophyly of Triticeae (Devos et al., 1993) and Andropogoneae (Wilson et al., 1999).

Phylogenetic analyses of individual molecular data sets within the last decade have converged on a set of well-supported relationships within Poaceae. Changes in the circumscriptions of subfamilies, and in the number of subfamilies recognized, clearly are necessary. The GPWG analyses presented here provide robust support for the major clades within the grass family, and provide the basis for the first family-wide subfamilial classification based on an explicit phylogenetic hypothesis.

Materials and Methods

Organization of the GPWG

The Grass Phylogeny Working Group was formed explicitly to combine available data on the phylogeny of the grass family and to use these data to propose a new classification. Most contributors had already published papers on grass phylogeny and were invited to contribute their data, both published and unpublished. Each contributor retained control over his or her data and was free to publish at any time, but the group agreed that the entire data set would be published as a single paper. Most of the collaboration has been conducted via e-mail, and the entire group has never met in a single place. This may serve as a model for future collaborations in plant systematics.

A list of taxa was drawn up in 1995 by LGC, JID, and EAK to improve parallel sampling for all data sets (Table 2; Appendix 1). This list was chosen to include as many of the major lineages in the family as possible, based on our knowledge from previous studies. Although sampling of taxa is still not perfectly parallel, many sequences were generated for this particular set of taxa. DNA was exchanged as necessary among members of the group. The list was expanded slightly based on results acquired during the study.

All sequences available at the end of 1997 were assembled by EAK into a single large data set in NEXUS format. The data set was then distributed via e-mail to all participants, who had the opportunity to comment on it. A "final" version of the data set was then distributed. Based on the results of the first round of analyses (GPWG, 2000), the

Table 2. Summary of genes and taxa included in the combined analysis. Taxa are listed approximately in the order in which they appear in Figure 1. cp rs = chloroplast restriction sites; GBS = GBSSI; struc. = structural data; * = composite taxon, represented by sequences from several genera; # = composite taxon, represented by sequences of different species within the same genus (as in Appendix I). For details of species, authorities, original publications, and GenBank accession numbers, see Appendix I. *Merxmuellera m.* = *Merxmuellera macowanii, Merxmuellera r.* = *Merxmuellera rangei.*

Genus	cp rs	*ndh*F	*phy*B	*rbc*L	*rpo*C	GBS	ITS	struc.
Flagellaria	X	X	X	X				X
Elegia#		X		X				X
Baloskion	X	X		X				X
Joinvillea#	X	X	X	X	X		X	X
Anomochloa	X	X	X	X		X		X
Streptochaeta#	X	X	X				X	X
Pharus#	X	X	X			X	X	X
Guaduella		X		X				X
Puelia		X	X	X				X
Eremitis	X	X	X			X		X
Pariana		X	X			X		X
Lithachne#	X	X	X	X			X	X
Olyra#	X	X	X		X			X
Buergersiochloa		X	X					X
*Pseudosasa**	X	X	X	X	X			X
Chusquea#	X	X	X	X		X	X	X
Streptogyna		X	X					X
Ehrharta#	X	X	X		X		X	X
Oryza	X	X	X	X	X	X	X	X
Leersia#	X	X		X			X	X
Phaenosperma		X						X
Brachyelytrum	X	X					X	X
Lygeum	X	X	X		X	X	X	X
Nardus	X	X	X		X		X	X
Anisopogon	X	X	X		X		X	X
Ampelodesmos	X	X					X	X
Stipa#	X	X		X	X		X	X
Nassella#	X	X	X				X	X
Piptatherum#	X	X					X	X
Brachypodium#	X	X	X				X	X
Melica#	X	X	X			X	X	X
Glyceria#	X	X	X			X	X	X
Diarrhena#	X	X	X				X	X
*Avena**	X	X	X	X	X		X	X
Bromus#	X	X	X	X	X		X	X
*Triticum**	X	X	X	X		X	X	X
Aristida#	X	X	X	X	X		X	X
Stipagrostis		X		X	X		X	X
Amphipogon#	X	X		X	X		X	X
Arundo	X	X		X	X		X	X
*Molinia**	X	X	X	X	X		X	X
Phragmites	X	X	X	X	X		X	X
Merxmuellera m.		X		X	X	X	X	X
Karroochloa		X		X	X	X	X	X
Danthonia#	X	X	X	X	X		X	X
Austrodanthonia		X		X	X	X	X	X
Merxmuellera r.		X		X	X	X	X	X
Centropodia		X		X	X	X	X	X
Eragrostis#	X	X	X	X	X		X	X
Uniola	X	X						X
Zoysia#	X	X						X

Table 2. Continued.

Genus	cp rs	*ndh*F	*phy*B	*rbc*L	*rpo*C	GBS	ITS	struc.
Distichlis	X	X						X
*Pappophorum**		X		X	X			X
Spartina#	X	X			X		X	X
Sporobolus#	X	X	X				X	X
Eriachne#				X			X	X
Micraira#		X			X		X	X
Thysanolaena		X	X	X	X		X	X
Gynerium		X		X	X		X	X
Chasmanthium#	X	X	X	X	X		X	X
Zeugites		X						X
Danthoniopsis#		X	X			X		X
Panicum#	X	X	X		X		X	X
Pennisetum#	X	X	X	X	X	X	X	X
*Miscanthus**	X	X	X	X	X	X	X	X
Zea		X	X	X	X	X	X	X

taxon list was expanded to include several more danthonioid taxa, and the matrix was recompiled by JID. Although all participants in the GPWG were invited to undertake data analyses and comment on the final version of matrices, abstracts, and text, this was not required. Thus the analyses and text of this paper reflect largely the work of LGC, JID, and EAK, with input from several members of the group. The GPWG website was created and is maintained by GFG.

TAXA

The taxa used in this analysis include four genera representing the families Flagellariaceae, Restionaceae (two genera), and Joinvilleaceae as outgroups. This choice was based on previous work (summarized in Kellogg & Linder, 1995) indicating that these represent the closest relatives of the grasses. The results of Briggs et al. (2000) suggest that the Ecdeiocoleaceae should be included in outgroup comparisons for the grasses in the future. Within the grass family, 62 exemplar species were chosen to represent the commonly recognized subfamilies Anomochlooideae, Arundinoideae, Bambusoideae, Centothecoideae, Chloridoideae, Ehrhartoideae (=Oryzoideae), Panicoideae, Pharoideae, and Pooideae, as well as species from several genera whose placement was uncertain (*Amphipogon*, *Anisopogon*, *Brachyelytrum*, *Buergersiochloa*, *Danthonia*, *Danthoniopsis*, *Eriachne*,

Table 3. Tree statistics for subsets of the data matrix. The percent missing data is the number of cells that are missing for the particular block when included in the total data matrix, and thus is equivalent to the number of missing taxa times the total number of informative characters plus missing data within sequences for scored taxa. Chloroplast r.s. = Chloroplast restriction sites.

Data set	# Taxa	Total # characters	# Inf. characters	% Missing data	Length	CI	RI	# Trees
Morphological	66	53	50	16.0	227	0.300	0.690	38,000+
Chloroplast r.s.	45	364	293	42.2	939	0.312	0.569	7
*ndh*F	65	2210	680	4.7	2894	0.379	0.582	16
phytochrome B	40	1182	417	45.5	1997	0.369	0.522	1
*rbc*L	37	1344	213	44.8	651	0.448	0.660	1
*rpo*C2	34	777	150	49.9	374	0.503	0.648	33
GBSSI	19	773	213	71.2	720	0.479	0.504	1
ITS	47	322	127	28.8	745	0.349	0.541	24
cp sequence data	66	4331	1043	25.2	3952	0.399	0.597	8
All cp data	66	4695	1336	26.5	4903	0.381	0.589	3
Nuclear	57	2277	757	51.9	3513	0.382	0.512	8
All molecular	66	6972	2093	33.6	8488	0.378	0.554	6
Combined data	66	7025	2143	33.2	8752	0.375	0.557	1

Table 4. List of structural characters and states. The first number in parentheses indicates the number of the same character in Soreng and Davis (1998), and the second number indicates the number of the same character in Kellogg and Campbell (1987); a "—" indicates that the character was not used in one or both of those analyses.

Culm

1 (1;—). Perennating woody culms: 0 = absent; 1 = present.
2 (2;—). Culm internodes: 0 = solid; 1 = hollow.

Leaf

3 (3;—). Leaf sheath margins: 0 = free; 1 = fused for at least 1/4 of length.
4 (4;—). Adaxial ligule type: 0 = membrane (with or without fringe of hairs); 1 = fringe of hairs only.
5 (5;—). Abaxial (contra–) ligule: 0 = absent; 1 = present.
6 (—;—). Leaf blade: 0 = absent; 1 = present.
7 (6;—). Pseudopetiole: 0 = absent; 1 = present.

Spikelet

8 (—;—). Floret with a structure identifiable as a palea, this recognized as present when a flower arises on a contracted axis above an enshrouding prophyll (or something like it), in the axil of a lemma: 0 = absent; 1 = present.
9 (—;—). Spikelet pairs: 0 = absent; 1 = present.
10 (7;—). Pedicel of spikelet: 0 = absent; 1 = present.
11 (8; 3, 4). Proximal female-sterile florets in female-fertile spikelets: 0 = absent; 1 = present.
12 (9; 6). Number of female-fertile florets per female-fertile spikelet: 0 = two or more; 1 = one.
13 (10;—). Awn or mucro on fertile or sterile lemma: 0 = absent; 1 = present.
14 (—;—). Number of awns: 1 = 1 awn; 2 = 13–23 awns (unique to *Pappophorum*); 3 = 3 awns.
15 (11;—). Awn attachment: 0 = terminal / subterminal; 1 = from a sinus; 2 = dorsal.
16 (12; 1). Disarticulation above glumes: 0 = absent; 1 = present.
17 (13;—). Germination flap in lemma: 0 = absent; 1 = present.

Flower

18 (14;—). Lodicules: 0 = absent; 1 = present.
19 (15; 7). Lodicule number: 2 = two; 3 = three.
20 (16;—). Fusion of anterior pair of lodicules: 0 = free; 1 = fused.
21 (17; 8). Distally membranous portion of lodicule: 0 = absent; 1 = present.
22 (18; 9). Lodicule vascularization: 0 = very faint to absent; 1 = prominent.
23 (19; 10). Inner whorl, posterior stamen: 0 = absent; 1 = present.
24 (19; 10). Inner whorl, anterior stamen pair: 0 = absent; 1 = present.
25 (19; 10). Outer whorl, anterior stamen: 0 = absent; 1 = present.
26 (19; 10). Outer whorl, posterior stamen pair: 0 = absent; 1 = present.
27 (—;—). Anthers tetrasporangiate, dithecal = 0; anthers bisporangiate, monothecal = 1.
28 (20;—). Styles fused at least at base: 0 = absent; 1 = present.
29 (21;—). Number of stigmas: 1 = one; 2 = two; 3 = three; 4 = four.
30 (22;—). Highest order of stigmatic branching present: 1 = simple (unbranched, or with branches composed of single elongate papillate receptive cells, or with very short branches composed of a few papillate receptive cells, but in the latter case the stigmas linear in outline); 2 = primary (branches well developed, composed of series of dispersed papillate receptive cells, with secondary branches absent or minimally developed, stigmas lanceolate or broader); 3 = secondary (secondary to tertiary branches well developed, branches composed of series of dispersed papillate receptive cells).
31 (23;—). Number of locules and ovules per pistil (all three families have one ovule per locule): 1 = one; 2 = two; 3 = three.

Embryogeny

32 (—;—). Haustorial synergids: 0 = absent; 1 = present.

Fruit and Embryo

33 (24; 11). Hilum: 0 = long-linear, > 1/3 length of grain; 1 = nonlinear, < 1/3 length of grain, elliptical or broader to punctiform.
34 (25;—). Embryo position and structure: 0 = embedded, simple; 1 = lateral, grass-type.
35 (26; 15). Embryo epiblast: 0 = absent; 1 = present.
36 (27; 16). Embryo scutellar tail: 0 = absent; 1 = present.
37 (28; 17). Embryo mesocotyl internode: 0 = negligible; 1 = elongate.
38 (29; 18). Embryonic leaf margins: 0 = meeting; 1 = overlapping.
39 (30;—). Endosperm lipid: 0 = absent; 1 = present.
40 (31;—). Endosperm starch grain syndromes: 0 = *Triticum*-type (simple grains only, dimorphic in size, round or lenticular, free); 1 = *Festuca*-type (highly compound grains present, with or without simple grains also present); 2 = *Andropogon*-type (simple and compound grains both present, the latter consisting of few granules); 3 = *Panicum*-type (simple grains only, uniform in size, small to medium, angular or sometimes smooth walled, densely packed); 4 = *Brachyelytrum*-type (simple only, large).

Seedling

41 (32; 20). Lamina of first seedling leaf: 0 = absent; 1 = present.

Table 4. Continued.

Vegetative Anatomy

42 (—;—). Differentiation of leaf epidermal cells into long and short (cork) cells: 0 = absent (i.e., cells ± undifferentiated); 1 = present (Campbell & Kellogg, 1987).

43 (34; 21). Multicellular microhairs: 0 = absent; 1 = present.

44 (35; 22). Occurrence in multicellular microhairs of a broad, short terminal cell, often with a longer basal cell, the walls of the terminal and basal cells similar in thickness: 0 = absent; 1 = present.

45 (36; 31). Arm cells: 0 = absent; 1 = present.

46 (37;—). Fusoid cells: 0 = absent; 1 = present.

Chromosomes

47 (—;—). Base chromosome number is same as state number except that 0 = 10; 1 = 11; 2 = 12; 3 = 13; 4 = 18; 5 = 19.

Biochemistry

48 (38; 30). Carbon fixation pathway: 0 = C_3; 1 = C_4 NADP-ME classical-type; 2 = C_4 NADP-ME *Aristida*-type; 3 = C_4 NAD-ME; 4 = C_4 NADP-ME Arundinelleae-type; 5 = C_4 NADP-ME *Eriachne*-type.

49 (39; 30). Carbon fixation PCK: 0 = absent; 1 = present.

Deletion in Phytochrome B

50 (—;—). 3-bp DNA deletion in phytochrome B: 0 = 3-bp DNA present (i.e., non-deleted state); 1 = DNA absent (i.e., deleted state; the deleted codon is at position 402 in the alignment of Mathews et al., 1995).

Chloroplast Genome Structure

51 (40;—). 6.4 kb inversion in the large single-copy region of the chloroplast genome, relative to the gene arrangement in *Nicotiana*: 0 = absent; 1 = present.

52 (41;—). *trn*T inversion in the large single-copy region of the chloroplast genome, relative to the gene arrangement in *Nicotiana*: 0 = absent; 1 = present.

53 (42;—). 15 bp insertion in *ndh*F at position 101951 of the chloroplast genome of *Oryza sativa*: 0 = absent; 1 = present.

Lygeum, Micraira, Nardus, Pariana, Phaenosperma, Puelia, Streptogyna, Thysanolaena).

For 31 of the terminal taxa in the matrix, all molecular data were taken from a single species; for an additional 27, data were from two or more species of the same genus (noted by # in Table 2: Appendix I). In eight cases, however, data from several genera were combined to create a "conglomerate" taxon (asterisks in Table 2). For example, although one listed representative of the Andropogoneae is *Miscanthus*, there is no *rbc*L sequence available for that genus. There is, however, a sequence for *Sorghum*. Thus the *Sorghum* sequence for *rbc*L was combined with the *Miscanthus* sequences for *ndh*F, creating a fictive taxon, an approach used previously by Kellogg and Linder (1995). This assumes that both genera are part of a monophyletic higher-level group (in this case, Andropogoneae, which are certainly monophyletic; Spangler et al., 1999). The results of such combinations are potentially misleading, in that they assume certain combinations of characters that may not ever actually occur in a single plant. We feel that the number of characters involved, however, is small, and the addition of phylogenetically informative characters by including the line of data outweighs the risk of misleading results. Any subsequent studies, particularly those for which there are more than two representatives of taxa combined here, should break up each conglomerate taxon into real species (i.e., exemplar taxa).

The number of taxa was dictated by the numbers of available sequences in the largest of the original data sets (*ndh*F and chloroplast restriction sites). Recent work on large phylogenies suggests that phylogenetic accuracy is improved by a very dense sample of taxa (e.g., Hillis, 1996, 1998; Graybeal, 1998). Producing a large data set with perfectly parallel sampling, however, would have required either a centralized effort in a single lab, or a formal, coordinated, and separately funded effort among multiple labs, rather than the decentralized approach used here.

CHARACTERS

The data matrix included 7025 characters assembled from the following sources:

1. NADH dehydrogenase, subunit F (*ndh*F)—Clark et al. (1995, 2000); Davis et al. (this paper); Spangler et al. (1999).
2. Ribulose 1,5-bisphosphate carboxylase/oxygenase, large subunit (*rbc*L)—Barker et al. (1995); Barker (1997); Doebley et al. (1990); Duvall and Morton (1996).
3. RNA polymerase II, β″ subunit (*rpo*C2)—Cummings et al. (1994); Barker et al. (1999).

4. Chloroplast restriction sites—Davis and Soreng (1993); Soreng and Davis (1998).
5. Phytochrome B (*phy*B)—Mathews and Sharrock (1996); Mathews et al. (2000).
6. Internal transcribed spacer of the nuclear ribosomal RNA (ITS)—Hsiao et al. (1998, 1999).
7. Granule bound starch synthase I (GBSSI, or *waxy*)—Mason-Gamer et al. (1998).
8. Morphology—Soreng and Davis (1998, and additional members of the GPWG, this paper).

Information on numbers of characters and taxa for each matrix is in Table 3, and the structural character list is in Table 4. The morphological (structural) matrix is in Appendix II. The first four data sets represent the chloroplast genome, and the next three the nuclear genome. Five data sets, *ndh*F, *rbc*L, *rpo*C2, *phy*B, and GBSSI, are all protein coding sequences; introns of GBSSI were not included in the alignments. The full data matrix included 66 taxa and 7025 characters, for a total of 463,650 cells. The amount of missing data for the total data set is 33.2% and varies among genes and taxa (Table 3). The full data matrix can be obtained from LGC, JID, EAK, or HPL, or from the GPWG website, or at TreeBASE (http://herbaria.harvard.edu/treebase/index.html).

Alignments were provided by the contributors except for the ITS data, for which the alignment was constructed by EAK, beginning with an initial alignment in ClustalW (Thompson et al., 1994) and then continuing by eye. It became apparent that ITS1 could not be aligned reliably across the family, so it was omitted from the data set. Later after extensive data exploration, several regions of ambiguous alignment were also omitted from ITS2. Gaps were treated as missing data. A few indels, identified as phylogenetically informative in analyses of individual data sets, were coded as binary characters and included in the structural data matrix (Appendix II).

DATA ANALYSIS

Data were analyzed by parsimony algorithms, as implemented in PAUP*4.0 d64 (Swofford, 1998) on a Power Macintosh G3, and Nona (Goloboff, 1993) on an Intel-chip-based workstation running Windows NT. Data sets were analyzed individually by JID, LGC, EAK, and HPL to be sure that e-mail transmittal of such a large file had not introduced any errors (for which we suggest the term "network-induced homoplasy"). Numbers of informative characters and tree lengths were the same for the two programs, although in some cases the number of equally parsimonious trees differed because of different ways the two programs count resolutions of polytomies.

PAUP* analyses used 10 random addition sequences, MULPARS on, TBR branch swapping, and MAXTREES set to automatically increase by 100. Bootstrap analyses (bts) used the full heuristic option, 500 or 1000 replicates. Bremer support (abbreviated here as brs; Bremer, 1988; Källersjö et al., 1992; also called decay index, cf. Donoghue et al., 1992) was also calculated. For tree lengths up to 11 steps longer than the shortest tree (up to 8763 steps), all trees were saved and the strict consensus computed. Because of memory limitations the method of negative constraints (Baum et al., 1994) was used to compute higher Bremer support values. The search for optimal trees was found to be quite inefficient with this method and often led to inflated support values. To minimize this problem, each search was done with 10,000 random addition sequences. Even so, the search frequently found trees shorter than the negative constraint tree, indicating that the previous searches had missed some trees. Computing Bremer support thus took almost two weeks of computer time on a G3. For the tree presented here, we arbitrarily chose a cut-off of 34 steps, so brs values above that are simply reported as "> 34."

To assess robustness of the results to choice of markers, each data set was analyzed by itself. The morphological data set was omitted from one analysis, and the chloroplast data were analyzed separately, as were the nuclear data. For analyses of individual data sets, PAUP* was set to perform heuristic searches using maximum parsimony, gaps were coded as missing data, multistate taxa were coded as uncertain, and starting trees were obtained by ten random addition sequences, holding one tree at each step; branch swapping used tree bisection and regrafting (TBR), steepest descent was not in effect, and MULPARS was in effect. Bootstrap analyses of individual data sets were done to facilitate comparisons with combined analyses. All bootstraps of individual data sets included 500 bootstrap replicates; MAXTREES was set to 500 to minimize times for searches.

Analyses conducted with Nona ver. 1.6 (Goloboff, 1993) used the default settings amb- (clades resolved only if they have unambiguous support) and poly= (polytomies allowed). Tree searches involved 1000 Wagner tree initiations using random taxon entry sequences, followed by tree bisection reconnection (tbr) swapping with up to 20 most-parsimonious trees retained in each search (hold/20, mult*1000); shortest trees retained from the subsearches were then tbr-swapped to completion,

with up to 10,000 trees held in memory (ho10000, max*). Structural character autapomorphies of terminals, and synapomorphies of clades, were determined by optimizing the morphological data on most-parsimonious trees obtained by the various analyses, using Winclada ver. 0.9.99m6.1 (Nixon, 2000). Strict-consensus bootstrap frequencies for just the total evidence analysis (see Soreng & Davis, 1998) were computed with Clados ver. 1.9.95 (Nixon, 1993) running Nona (Goloboff, 1993) as a daughter process for the tree searches, using a copy of the data set from which uninformative characters had been removed (with the "mop" function of Winclada). One thousand bootstrap replicates were conducted, using the same ambiguity and polytomy settings as in the basic analyses. Each replicate consisted of 10 random taxon entry sequences followed by tbr swapping with up to 10 trees retained from each subsearch (ho/10, mult*10), and with further tbr swapping then conducted on the resulting trees from the 10 subsearches, with 101 trees held (ho 101, max*).

Uninformative characters were excluded for all analyses, so all tree statistics reported in this paper (consistency index [CI] and retention index [RI]) reflect only potentially phylogenetically informative characters.

Chloroplast and nuclear trees were compared using the incongruence length difference test (random partition test of Farris et al., 1994), as implemented in PAUP*. They were also compared using simple inspection, as recommended by Mason-Gamer and Kellogg (1996). To compare tree topologies, constraint trees were constructed as necessary in MacClade (Maddison & Maddison, 1993); these were then loaded, the constraint enforced, and a heuristic search undertaken using the same parameters as in unconstrained searches.

The combined data were constrained to fit topologies suggested in previous studies by loading a constraint tree in PAUP* and then searching for the most parsimonious tree compatible with that constraint tree. Constrained and unconstrained trees were compared using the Wilcoxon signed ranks test (WSR) as suggested by Templeton (1983) and implemented by Mason-Gamer and Kellogg (1996). Significance values were determined using a two-tailed test.

The entire data set could not be analyzed with neighbor-joining or maximum likelihood algorithms. The inclusion of morphological and restriction site data with sequences made it nonsensical to specify a single model of evolution. While a model could in principle be hypothesized for morphological or restriction site data, it would have to be different from the model specified for sequence data. Calculation of base frequencies and transition/transversion ratios would be meaningless. Several neighbor-joining analyses were done with morphological and restriction site data omitted, but this also required omitting several taxa for which distances were then undefined because of missing data. By the time data sets and taxa were omitted, the results were difficult to compare to those of parsimony algorithms. Several maximum likelihood analyses were also undertaken on sequence data alone. These did not reach completion even after three to five days of analysis time. As with the neighbor-joining analyses, missing data and different models of evolution for the different genes made the results of questionable validity.

Results

Consensus trees for analyses of the individual data sets are presented in Appendix III-A to H and tree statistics in Tables 3 and 5. Note that the taxa included are generally selected from more comprehensive analyses that have been published elsewhere, as described in Materials and Methods. Many of the trees differed in topology, but in no case was a strongly supported group in one tree contradicted by a strongly supported group in another tree. We interpreted this as lack of significant conflict. Nonetheless, the ILD test indicated significant differences between the nuclear and chloroplast data sets, between nuclear protein-coding and chloroplast, between *ndh*F and *phy*B, and between *ndh*F and *rbc*L. These differences persisted in most cases even when taxa with conflicting placements were removed. In the only exception to this observation, *ndh*F and *phy*B were not significantly different if the PACCAD Clade was reduced to Panicoideae, Chloridoideae, and the clade of *Molinia* plus *Phragmites*. This provides weak evidence that differences in resolution of the PACCAD Clade (Panicoideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae) are partly responsible for the significant differences. Differences between *ndh*F and *rbc*L, however, are puzzling because both are part of the same linkage group. Because of the ambiguity of the results, we did not attempt to do all possible pairwise comparisons of trees. Despite the differences in the data sets, we chose to combine the data in a single analysis. Different histories for the various genes remain a formal possibility. However, in other investigations we have seen that the ILD test may return significant differences if there is extensive missing data (as we have in some data sets here)

Table 5. Bootstrap support values for subsets of the total data matrix. Numbers of nodes at particular support values are given as fractions of the total number of nodes, and also as decimals. Poly = polyphyletic; para = paraphyletic. Anom. = Anomochlooideae; Phar. = Pharoideae; Puel. = Puelioideae. *Panicoideae here include *Danthoniopsis*; if it is excluded then support values are much higher.

	Total data	Molec. data	Chloro-plast	Nuclear	Structural	*ndh*F	cprs	*rbc*L	*rpo*C2	*phy*B	GBSSI	ITS
# Nodes 100	27/64	25/64	22/64	2/55	0	23/63	2/43	8/35	2/32	4/38	3/17	1/45
	(0.42)	(0.39)	(0.34)	(0.04)		(0.36)	(0.05)	(0.23)	(0.06)	(0.11)	(0.18)	(0.02)
# Nodes 90–99	9/64	14/64	14/64	12/55	1/64	11/63	8/43	7/35	6/32	13/38	4/17	5/45
	(0.14)	(0.22)	(0.22)	(0.22)	(0.02)	(0.17)	(0.19)	(0.20)	(0.19)	(0.34)	(0.24)	(0.11)
# Nodes 70–89	11/64	8/64	7/64	5/55	4/64	4/63	9/43	6/35	4/32	10/38	1/17	4/45
	(0.17)	(0.13)	(0.11)	(0.09)	(0.06)	(0.06)	(0.21)	(0.17)	(0.12)	(0.26)	(0.06)	(0.09)
Fraction nodes > 70	0.73	0.73	0.67	0.35	0.08	0.59	0.45	0.60	0.37	0.71	0.48	0.22
Poaceae	100	100	100	97	Para	100	98	99	Not tested	96	Not tested	Not tested
Spikelet Clade	100	99	98	87	Para	94	81	98	Not tested	54	Not tested	83
Bistigmatic Clade	100	100	100	97	<50	100	<50	Not tested	Not tested	79	100	98
BEP + PACCAD Clade	100	100	99	81	Poly	100	Not tested	Para	Not tested	75	Not tested	Not tested
Bambusoideae	97	98	97	Para	Poly	100	Para	Para	Para	92	Poly	75
Ehrhartoideae	100	100	100	92	Para	98	72	100	51	99	Not tested	54
Pooideae	100	100	93	95	Para	88	<50	70	88	94	77	19
BEP	71	90	62	50	Para	53	Para	Para	56	89	Poly	Para
Aristidoideae	100	100	100	84	84	100	Not tested	98	67	Not tested	Not tested	76
Chloridoideae	86	83	86	51	Para	<50	98	54	63	93	95	Poly
Panicoideae*	65	Poly	Para	63	Para	Poly	Para	95	93	99	61	Poly
Danthonioideae	98	97	98	Poly	Para	99	Not tested	83	70	Not tested	Poly	Poly
PACC	100	100	99	77	<50	100	95	73	73	90	Poly	<50
Arundinoideae s. str.	77	78	<50	Poly	Para	<50	Not resolved	23	Poly	52	Not tested	Poly
Centothecoideae	Para	Poly	Para	Para	Poly	Para	Not tested	64	Para	98	Not tested	Para

or if a single terminal taxon differs in its placement (Z. Magombo, pers. comm.). Because of the lack of obvious points of conflict between the data sets, and because of the clear congruence at the deep nodes with which we are concerned, we interpret the significant ILD tests as misleading.

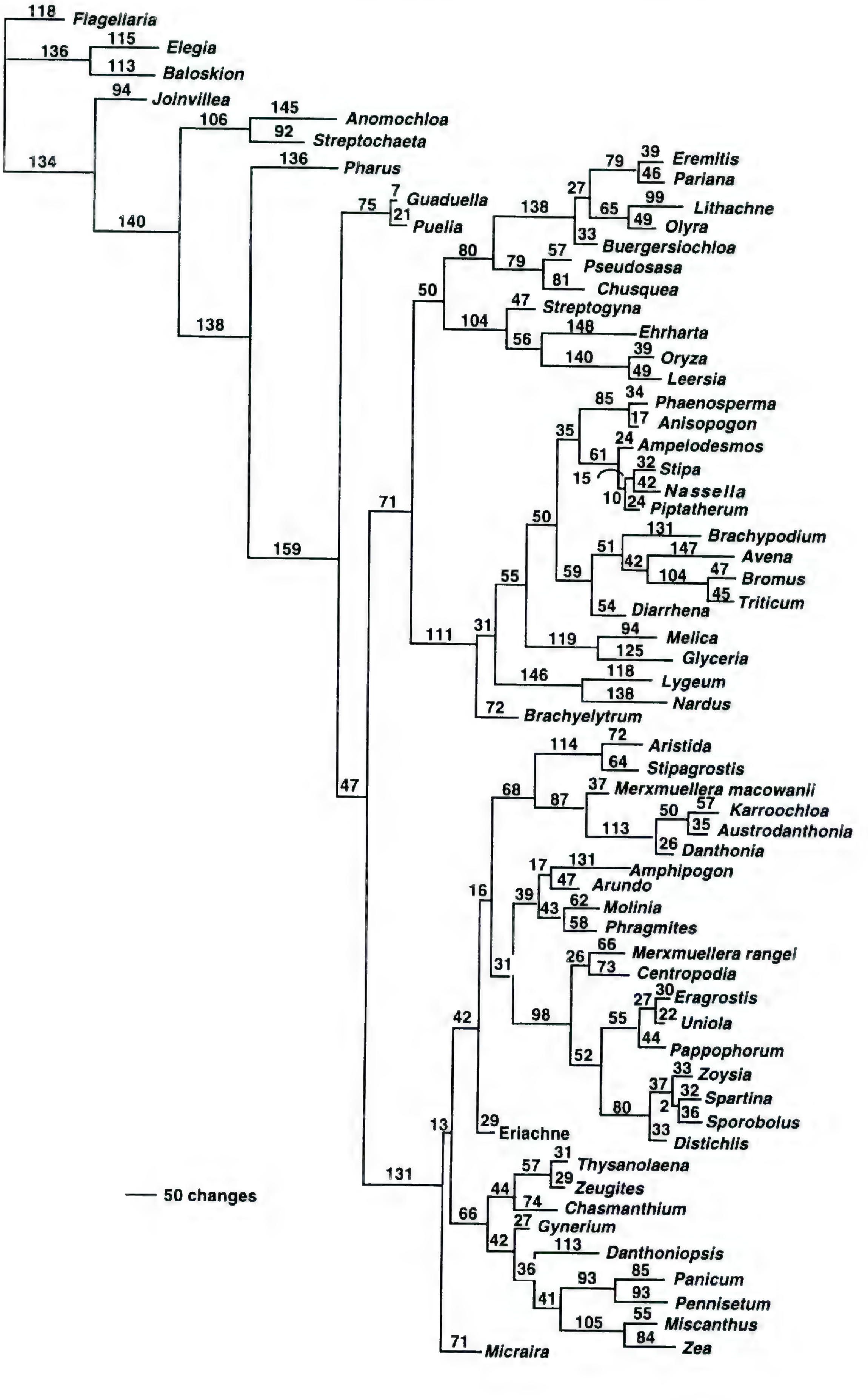
Analyses of the complete data set were faster than analyses of many of the individual data sets, as has been found in studies of other large data sets (Soltis et al., 1998). For example, a heuristic search of the complete data set in PAUP* on a Macintosh G3 with 10 random addition sequences took 19.6 seconds.

With all data combined, there were 2143 parsimony informative characters, which produced a single tree of 8752 steps, consistency index (CI) of 0.375, and retention index (RI) of 0.557 (Figs. 1 and 2). Bootstrap analyses (1000 replicates) indicated that 27 branches were supported in 100% of the bootstrap replicates, 9 branches in 90–99%, and 11 branches in 70–89% (Table 5). Put another way, of 64 internal nodes, slightly more than half (36) have bootstrap values over 90% and a clear majority (47) have values over 70%. Bootstrap values were virtually identical whether done using strict consensus bootstrap in Nona (Goloboff, 1993) or the frequency-within-replicates bootstrap in PAUP*4.0 (Swofford, 1998); for comparison with individual analyses, we report the values from PAUP*4.0.

The analysis of the combined data confirms many results of previous studies and clarifies some relationships that were previously ambiguous. The two species of Restionaceae form a clade. *Joinvillea* is sister to a monophyletic Poaceae. The three earliest diverging lineages are the Anomochlooideae, Pharoideae, and Puelioideae, in that order, together accounting for 30 species of grasses. The vast majority of extant grasses fall into two distinct lineages. One of these is the PACC clade (Davis & Soreng, 1993), here called the PACCAD Clade (Panicoideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae) to reflect the inclusion of two additional subfamilies within the clade. Within this clade, Panicoideae s. str. (excluding *Danthoniopsis*) are monophyletic (bts 94; brs 10), as are the core Paniceae sampled here (bts 100; brs 25) and Andropogoneae (bts 100; brs 32). Other strongly supported groups in the PACCAD Clade correspond to Aristidoideae (bts 100; brs 25) and Danthonioideae (bts 98; brs 15). The traditional Chloridoideae are supported at bts 99 (brs 16), and the clade including the chloridoids plus *Centropodia glauca* and *Merxmuellera rangei* (Chloridoideae s.l.) is also reasonably well supported at bts 86 (brs 8). A clade corresponding to Arundinoideae s. str.—*Arundo*, *Amphipogon*, *Molinia*, and *Phragmites*—receives modest support from this analysis (bts 77, brs 6), but the sister relationship of *Molinia* and *Phragmites* is well supported (bts 100; brs 16). The other major clade (the BEP Clade) is less well supported (bts 71; brs 8) and includes Bambusoideae s. str., Ehrhartoideae (= Oryzoideae), and Pooideae. Bambusoideae are monophyletic (bts 97; brs 15), as is the clade including the herbaceous bamboos (bts 100; brs 18). Likewise Ehrhartoideae are monophyletic (bts 100; brs 24), as are Oryzeae (bts 100; brs > 34). Pooideae include *Brachyelytrum* (bts 100; brs 15), and most nodes within the pooid clade are strongly supported.

Despite the strong phylogenetic pattern shown by the combined analysis, placement of some taxa remains ambiguous. The major uncertainty remains the monophyly of the BEP Clade. As noted earlier (GPWG, 2000), it is almost equally parsimonious to place Pooideae as sister to the PACCAD Clade, and this makes evolution of particular morphological characters more parsimonious. The Pooideae plus PACCAD group appears in analyses of *rbc*L (Appendix III-C), chloroplast restriction sites (Appendix III-A), morphology (Appendix III-H), and ITS (Appendix III-F), whereas the BEP Clade is retrieved by analyses of *ndh*F (Appendix III-B), *rpo*C2 (Appendix III-D), and *phy*B (Appendix III-E). GBSSI (Appendix III-G) forms a novel topology, in which neither the PACCAD nor the BEP Clades is monophyletic. An analysis combining *rbc*L, chloroplast restriction sites, ITS, and morphology retrieves, not surprisingly, a clade that links the Pooideae with the PACCAD Clade. Bootstrap analysis, however, finds that the Pooideae + PACCAD clade occurs in only 23% of the replicates, although it appears in 40% if *Streptogyna* is considered part of the clade. The BEP Clade was not found in any of the bootstrap partitions.

Constraining the entire data set to place Pooideae sister to the PACCAD Clade resulted in a single tree eight steps longer than the most parsimonious tree. The net change of eight steps, however, was produced by changes of one or two steps in 107 characters from throughout the data set. A Wilcoxon signed rank test (Templeton, 1983; Mason-Gamer & Kellogg, 1996) resulted in a test statistic of 2654; for $n = 107$, this corresponds to $p < 0.406$ (two-tailed test). This means that we cannot rule out the possibility that Pooideae are indeed sister to the PACCAD Clade. This is true even if the morphological characters are excluded ($z = 1.146$; $P < 0.254$).


Flagellaria
Elegia
Baloskion
Joinvillea
Anomochloa
Streptochaeta
Pharus
Guaduella
Puelia
Eremitis
Pariana
Lithachne
Olyra
Buergersiochloa
Pseudosasa
Chusquea
Streptogyna
Ehrharta
Oryza
Leersia
Phaenosperma
Anisopogon
Ampelodesmos
Stipa
Nassella
Piptatherum
Brachypodium
Avena
Bromus
Triticum
Diarrhena
Melica
Glyceria
Lygeum
Nardus
Brachyelytrum
Aristida
Stipagrostis
Merxmuellera macowanii
Karroochloa
Austrodanthonia
Danthonia
Amphipogon
Arundo
Molinia
Phragmites
Merxmuellera rangei
Centropodia
Eragrostis
Uniola
Pappophorum
Zoysia
Spartina
Sporobolus
Distichlis
Eriachne
Thysanolaena
Zeugites
Chasmanthium
Gynerium
Danthoniopsis
Panicum
Pennisetum
Miscanthus
Zea
Micraira
50 changes

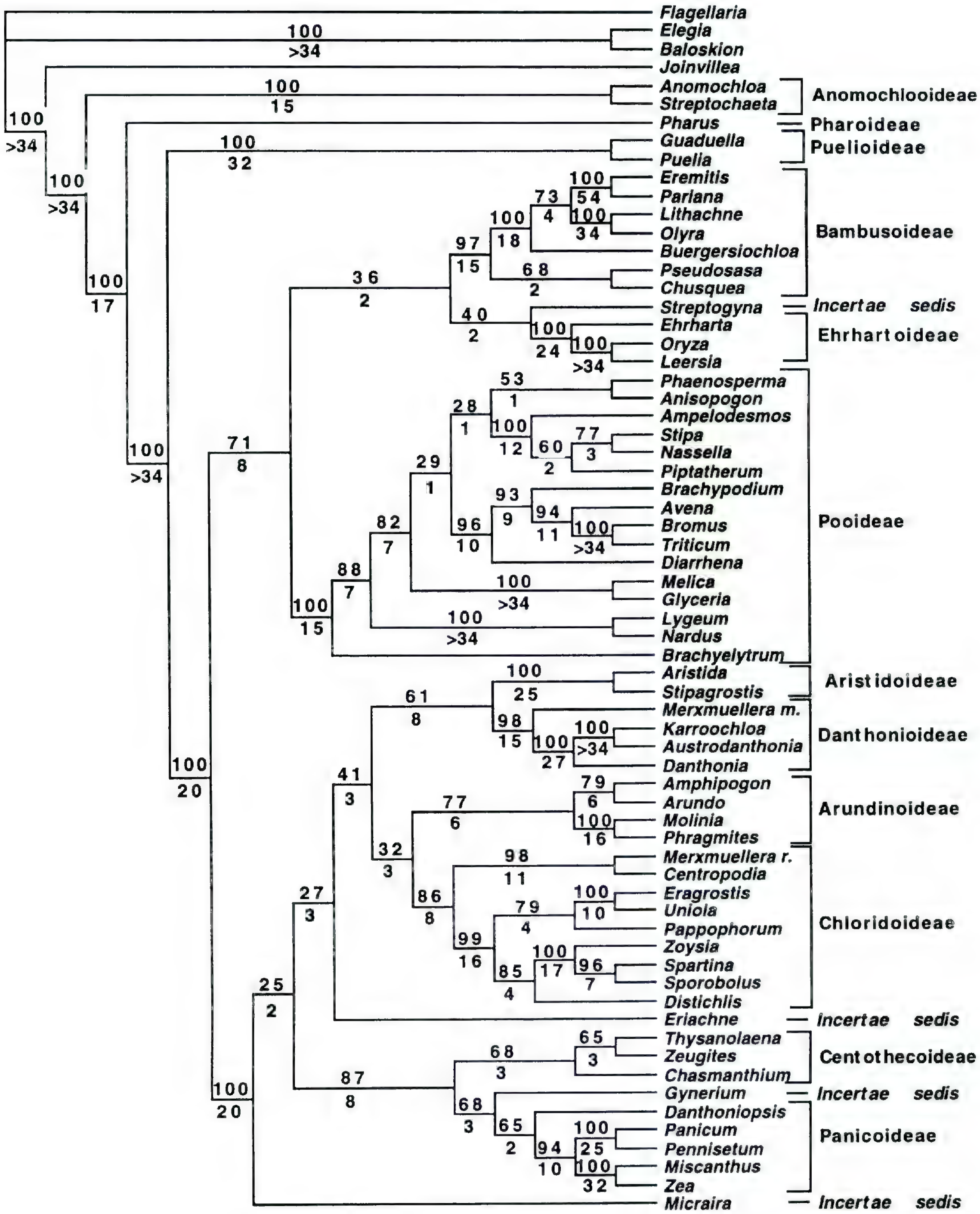


Figure 2. Same tree as Figure 1, showing percent of bootstrap replicates above lines and Bremer support below. Brackets indicate the revised classification for the Poaceae.

←

Figure 1. Single most parsimonious tree for the grasses and relatives, based on eight sets of data. Length = 8752 steps, CI = 0.375, RI = 0.557. Numbers above branches are numbers of unambiguous changes. Branches are drawn proportional to length.

The combined analysis places *Streptogyna* as sister to Ehrhartoideae, but this result is not strongly supported (bts 40; brs 2). This partly reflects missing data, in that only *ndh*F and *phy*B sequences are available for *Streptogyna*, in addition to morphological data. *ndh*F places *S. americana* sister to Ehrhartoideae, whereas *phy*B places it as sister to the entire BEP Clade, and morphological data fail to resolve its position.

The combined data place the woody bamboos, *Pseudosasa* and *Chusquea*, in a clade (bts 68; brs 2), as would be expected from previous studies (Zhang, 2000; Zhang & Clark, 2000). The pair appears monophyletic in chloroplast restriction site, morphological, and *phy*B trees. The two are paraphyletic, however, in trees using *rbc*L and *ndh*F, although this result is not well supported in these trees. "*Pseudosasa*" is a composite taxon, made up of data from several different genera, and this may also affect its placement in the combined tree.

Phaenosperma and *Anisopogon* are clearly members of the expanded pooid clade, where they are placed by all data sets, either singly or in combination. Their position within the clade, however, remains uncertain. They are sister taxa when all data are combined, but this result is not strongly supported (bts 53; brs 1); together they are sister to the Stipeae, also a poorly supported result (bts 28; brs 1). In *ndh*F, chloroplast restriction site, and *phy*B trees, *Anisopogon* is placed on a branch that diverges after the *Lygeum* + *Nardus* clade, but before the rest of the Pooideae (i.e., Stipeae, Meliceae, *Diarrhena*, *Brachypodium*, Aveneae, and Poeae). ITS places it sister to Aveneae/Poeae, and *rpo*C2 places it sister to *Stipa*. In no case is the placement strongly supported. The position of *Phaenosperma* is based only on *ndh*F and morphological data, and the latter are largely uninformative about its position.

Meliceae are monophyletic in all gene trees and in the combined tree. Their position, however, varies among the individual gene trees. The combined tree provides good evidence that Meliceae diverged after *Lygeum* + *Nardus* (bts 82; brs 7), but evidence is weak that it was the next diverging branch (bts 29; brs 1). Other possible placements include sister to Stipeae (*ndh*F, *phy*B), sister to *Diarrhena* + *Brachypodium* + Aveneae/Poeae (cp restriction sites), sister to a clade of *Brachypodium* + *Brachyelytrum* + (*Lygeum* + *Nardus*) (ITS), or paraphyletic at the base of the Pooideae (GBSSI). The ambiguity cannot be ascribed to missing data, although additional sampling among early-diverging Pooideae might be warranted.

The positions of *Eriachne* and *Micraira* are not firmly resolved by the combined data set, although both are clearly members of the PACCAD Clade. This almost certainly reflects missing data. In addition to morphological data, *Eriachne* is represented only by *rbc*L and ITS sequences, and *Micraira* by *ndh*F, *rpo*C2, and ITS. They are both isolated taxa, and in individual analyses fall at the base of other well-supported clades. The position of *Micraira* as sister to the entire PACCAD Clade appears only in the combined analysis, and likewise the position of *Eriachne* as sister to the Arundinoideae s. str. + Chloridoideae + Aristidoideae + Danthonioideae clade is both novel and poorly supported. Bootstrap analysis of the combined data set placed *Eriachne* and *Micraira* as sisters in 51% of the 1000 replicates, a position not supported by the most parsimonious tree.

Aristidoideae and Danthonioideae are both clearly monophyletic, and each is strongly supported by both bootstrap and decay analyses (bts 100 and 98; brs 25 and 15, respectively). In the combined tree they appear as sister taxa. The aristidoid/danthonioid clade is not strongly supported, however (bts 61; brs 8), and is reflected only in the *phy*B tree. *rbc*L places Aristidoideae sister to Chloridoideae, whereas *ndh*F and chloroplast restriction sites put Aristidoideae sister to the rest of the PACCAD Clade, and ITS places the subfamily sister to *Amphipogon* + Chloridoideae. *rpo*C2 indicates that Aristidoideae is derived from within Arundinoideae. *ndh*F places Danthonioideae sister to Panicoideae + Centothecoideae, whereas chloroplast restriction sites do not resolve the position of *Danthonia*. GBSSI retrieves a novel arrangement in which Danthonioideae are polyphyletic, but this result is not strongly supported and is likely affected by skewed taxon sampling in the GBSSI data set. *rpo*C2 suggests that Danthonioideae are sister to *Amphipogon*.

The relationships of *Zeugites*, *Thysanolaena*, *Chasmanthium*, *Danthoniopsis*, and *Gynerium* to each other and to the Panicoideae are not resolved by this analysis. The entire group is well supported as monophyletic (bts 87; brs 8), but other relationships are less clear. Only morphological and *ndh*F data are available for *Zeugites*, so its placement may be affected by missing data.

The morphological data have little effect on the analysis. When they are omitted, 6 trees are found in two islands (length 8488, CI = 0.378, RI = 0.554). The topology of the strict consensus (Appendix III-K) is similar to that of the combined tree except for the position of *Zeugites*, which is sister to *Danthoniopsis*, and *Gynerium*, which is sister to Panicoideae. In the consensus of the six trees, the

relationship of *Pseudosasa* and *Chusquea* is unresolved, as is the relationship of *Phaenosperma* and *Anisopogon*, and the position of Meliceae in the pooid clade. These are areas that were poorly supported even in the combined tree, and thus already known to be ambiguous. The most notable difference is the increased support for the BEP Clade, which is supported at a bootstrap value of 90%. The number of nodes with support greater than 90% (Table 5) is somewhat greater without the structural data, but the overall consistency index is not changed appreciably (Table 3).

The results for the entire data set largely reflect the results for the chloroplast data alone. The chloroplast data contribute 1336 potentially phylogenetically informative characters, or about 62% of the total. Analysis of these data alone produces 2 trees (length = 4903, CI = 0.381, RI = 0.589) that differ only in the relative positions of *Phaenosperma* and *Anisopogon* (Appendix III-I). The numbers of strongly supported nodes are about the same as for the entire data set (Table 5), and differences between the chloroplast tree and the entire data set are all in poorly supported areas of the tree (see below).

The chloroplast tree is only slightly affected by mixing sequence data with restriction site data. If the restriction site data are excluded so that the data set consists only of *ndh*F, *rbc*L, and *rpo*C2 data, the tree is virtually identical to the chloroplast tree except that *Pseudosasa* plus *Chusquea*, and *Phaenosperma* plus *Anisopogon* form monophyletic pairs rather than being paraphyletic (not shown). *Piptatherum* and *Nassella* are paraphyletic rather than sisters, and the Meliceae are sister to the core Pooideae rather than to the Stipeae.

Analysis of only the three nuclear genes (*phy*B, GBSSI, and ITS) required elimination of nine taxa for which nuclear data were not available. The analysis thus included 57 taxa and 757 characters and found eight trees (length = 3513, CI = 0.382, RI = 0.512) on one island (Appendix III-J). The nuclear trees were not as well supported as the chloroplast tree or the tree for the entire data set, which presumably reflects extensive missing data for GBSSI, and a generally smaller number of informative characters. Only two nodes were supported in 100% of the 1000 bootstrap replicates, and 11 had values between 90 and 99%.

Analysis of chloroplast data plus the data from the two protein-coding nuclear genes (that is, excluding morphological and ITS data) has little effect on either topology or support for the tree, perhaps because omitting morphology and ITS only eliminates 178 characters, or about 8% of the total. Differences appear only in the placement of the Meliceae, *Eriachne*, *Micraira*, *Zeugites*, and *Danthoniopsis*, all poorly supported areas of the trees.

Discussion

Well-supported clades

Some relationships appear consistently in all analyses of all data sets and are strongly supported by the combined analysis. Among these are the following (in order from the bottom of the tree):

1. Joinvilleaceae are sister to Poaceae.
2. Poaceae are monophyletic.
3. The earliest diverging lineage of Poaceae is Anomochlooideae (even if *Anomochloa* and *Streptochaeta* prove to be two separate lineages, they would still be the two earliest-diverging lineages in the family).
4. The next diverging lineage is Pharoideae.
5. The next diverging lineage is Puelioideae.
6. All remaining grasses form a clade, which appears to have diversified well after the origin of the family.
7. Bambusoideae s. str., Ehrhartoideae, Pooideae s.l., Aristidoideae, Danthonioideae, Arundinoideae s. str., Chloridoideae s. str., Chloridoideae s.l., and Panicoideae are all monophyletic.
8. Bambuseae, Parianeae, Olyreae s. str., Oryzeae, Stipeae, Meliceae, and *Lygeum* + *Nardus*, and *Molinia* + *Phragmites* are all monophyletic.
9. The PACCAD Clade—now including Panicoideae, Arundinoideae s. str., Chloridoideae s.l., Centothecoideae, Aristidoideae, Danthonioideae, *Eriachne*, *Micraira*, and *Gynerium*—is monophyletic.

As noted in the introduction, all of these relationships have been supported by previous studies and none is unique to the combined analysis. Previous studies, however, were limited because they were based on a single gene, a modest number of morphological characters, and/or a restricted sample of taxa. Because of the strong support for the relationships found in the present study, we propose a revised subfamilial classification (see Taxonomic Treatment). The revisions primarily reflect changes in circumscriptions of the Bambusoideae and Arundinoideae and involve only a small fraction of the species in the family. Over three quarters of the species are included in the subfamilies Pooideae, Chloridoideae, and Panicoideae, the circumscriptions of which are changed only slightly by the revisions.

Molecular characters

Virtually all of the phylogenetic signal in this analysis comes from the molecular data (Appendix

III-K), as expected. The combinable component consensus (Bremer, 1990) of the molecular trees (Kellogg, 1998) is remarkably well resolved; all nodes found in this consensus are strongly supported in the combined analysis presented here. When the molecular data are analyzed alone, all strongly supported nodes from the combined analysis are recovered, and support for the BEP Clade is increased.

Previous theoretical (Graybeal, 1998) and empirical (Soltis et al., 1998) studies have indicated that large numbers of characters may be necessary to resolve phylogenetic patterns, a conclusion only partially supported by this study. The molecular data alone and the entire data matrix included 2093 and 2143 phylogenetically informative characters, respectively (Table 3). These data sets found the largest percentage of nodes with bootstrap values above 70% (0.73 in both cases) but did not have the highest consistency or retention indices. The highest CI was produced by the *rpo*C2 data alone (150 informative characters), although this could be in part an artifact of alignment (see below), and the highest RI by the *rbc*L data alone (213 informative characters). The fraction of nodes with bootstrap values over 70% was almost as high for the phytochrome B data alone (with 417 informative characters) as for all data combined. We conclude that, while large numbers of informative characters may provide increased reliability, small numbers are not necessarily misleading or inaccurate samples of the whole.

Other studies have shown that the number of taxa included may affect phylogenetic accuracy (e.g., Hillis, 1996, 1998; Graybeal, 1998), although this is not necessarily the case (Poe & Swofford, 1999). Certainly future studies should include more taxa than just the set of exemplars used here. However, the results numbered 1 to 9 above have been found in analyses of virtually every individual data set, as well as in the combined tree, and we would be surprised if they were overturned by inclusion of more taxa.

Most of the molecular data come from chloroplast genes, so it is not surprising that the tree from the chloroplast alone closely matches the tree for the entire data set. The *ndh*F data set is missing the least data (Table 3) and has the most informative characters of the molecular data sets, presumably because it is the longest molecule. Our results confirm the utility of this molecule for resolving relationships among grass genera (Clark et al., 1995; Giussani et al., in press).

Alignment is a particular problem for the *rpo*C2 and ITS data used here. Alignment of the ITS data was difficult and confirmed our suspicion that it may not be useful at this level of divergence. ITS1 and parts of ITS2 had to be eliminated because of difficulty in assessment of sequence similarity. The *rpo*C2 sequences used here code for repeated amino acid motifs inserted into the protein. The insertion appears only in the grasses and thus constitutes a synapomorphy for the family (Cummings et al., 1994), although we did not code it as such in this analysis. The repeats are similar but not identical to each other in sequence, making alignment problematical, a point discussed at length by Barker (1995) and Barker et al. (1999). Efforts to improve alignments necessarily reduce apparent homoplasy; this may result in the high CI mentioned above. Although phylogenetic results from these molecules are similar to those from the other genes, by themselves the two sets of sequences do not permit confident assessments of relationships among subfamilies.

Trees from *ndh*F and *phy*B, individually, are particularly well resolved and well supported. Their congruence in early-diverging branches contributes to the strength of the overall topology of the combined data. In particular, *phy*B provides considerable support for the BEP Clade, a topology that is only weakly supported by *ndh*F, and not at all by several other data sets. The two data sets do appear to conflict in relationships among members of the PACCAD Clade, and this may be an area for future investigations.

As noted in Methods, sequences for a given genus were in some cases taken from different, congeneric species. This procedure assumes that the genus is monophyletic, an assumption that is almost certainly correct in many cases (e.g., *Joinvillea*, *Streptochaeta*), and perhaps not as likely in others. For example, the three species of *Stipa* sampled here have been placed in the genera *Achnatherum*, *Stipa*, and *Jarava* (Barkworth & Everett, 1987; Barkworth, 1993; Jacobs & Everett, 1997), which are distinct and possibly not a monophyletic group within Stipeae (Jacobs et al., 2000). While this problem is not likely to compromise our conclusions regarding subfamily relationships, it means that relationships among species of the Stipeae (or other tribes or genera where composite terminal taxa were used) cannot be addressed by this analysis.

STRUCTURAL CHARACTERS

The structural characters, comprising the morphological data set (Table 4), were optimized on the phylogeny (Fig. 3). Our results suggest that some

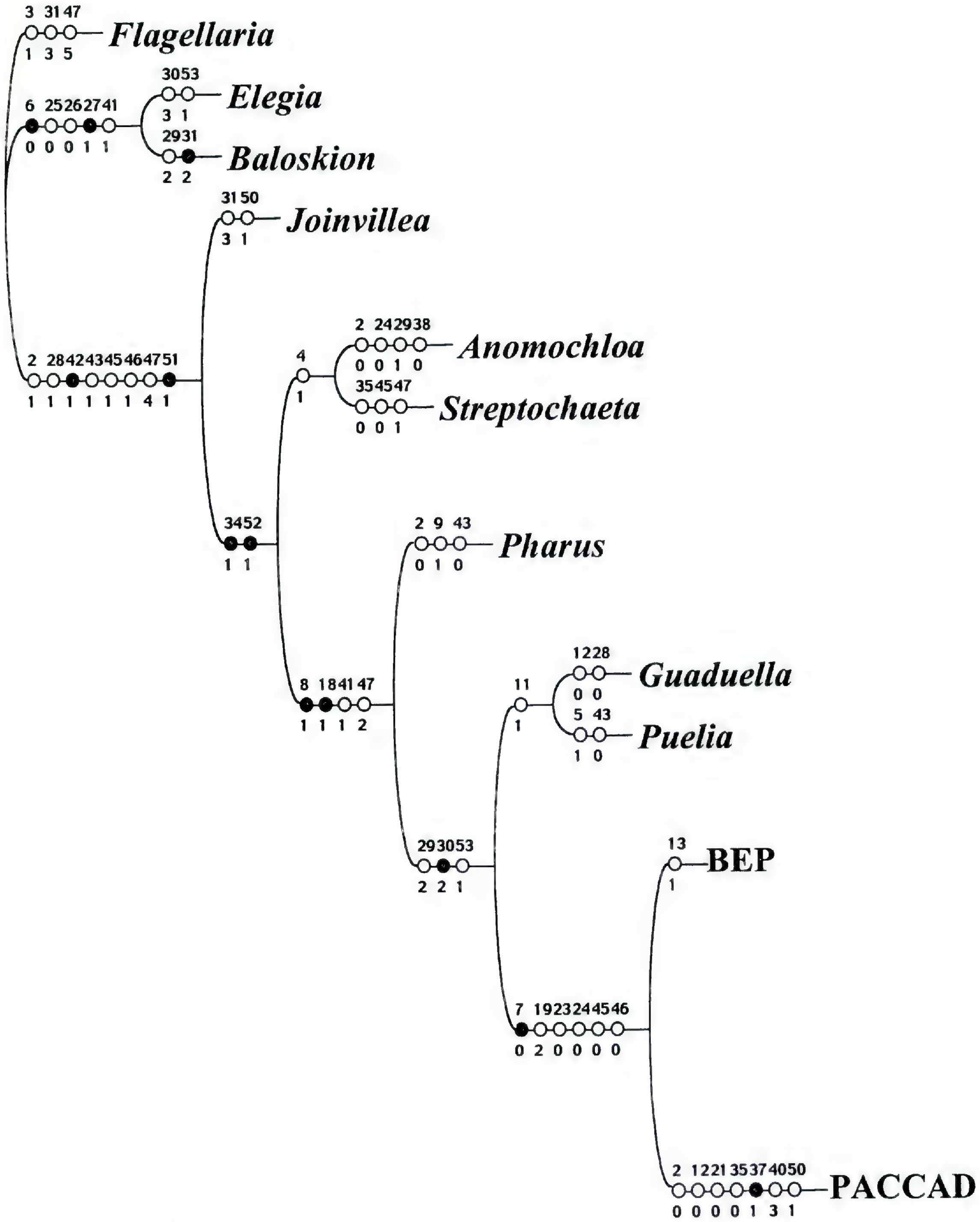


Figure 3 (pp. 395–397). Same tree as Figure 1, with structural characters mapped on using ACCTRAN optimization. Character number is above the branch, and the state to which the character changes is below. Filled circles represent unique occurrences of character states; open circles represent homoplasies.

of the morphological characters may be useful for delimiting groups within tribes or subfamilies, but are too variable to be useful in delimiting subfamilies; these include 2 (culms hollow or not), 3 (leaf sheath margins fused or free), 10 (pedicel present or not), 11 (presence or absence of proximal reduced flowers), 12 (number of flowers per spikelet), 13 (presence or absence of awns), 15 (attachment of awns), 16 (disarticulation above or below glumes), 20 (lodicule fusion), 28 (style fusion), 29

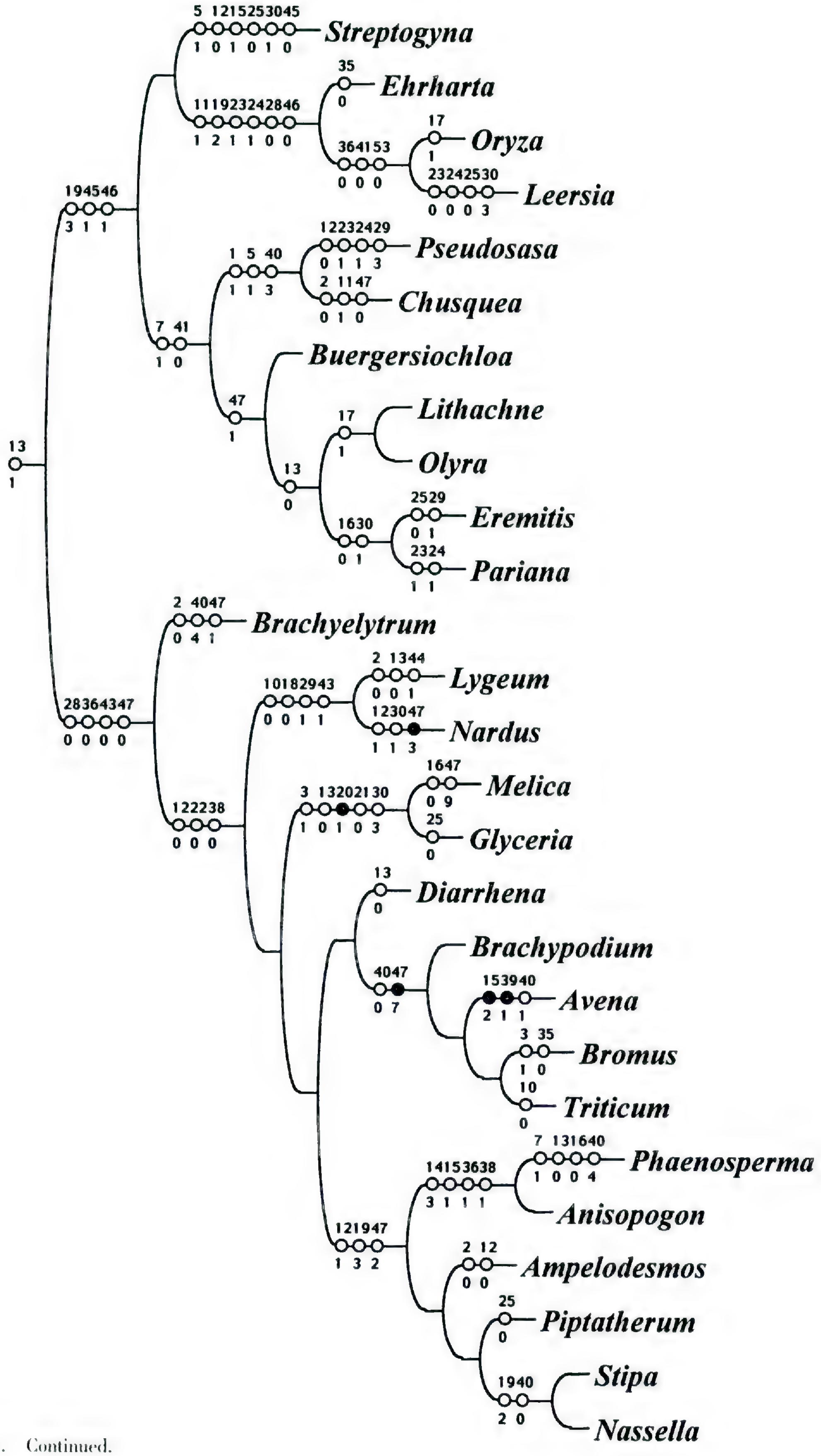


Figure 3. Continued.

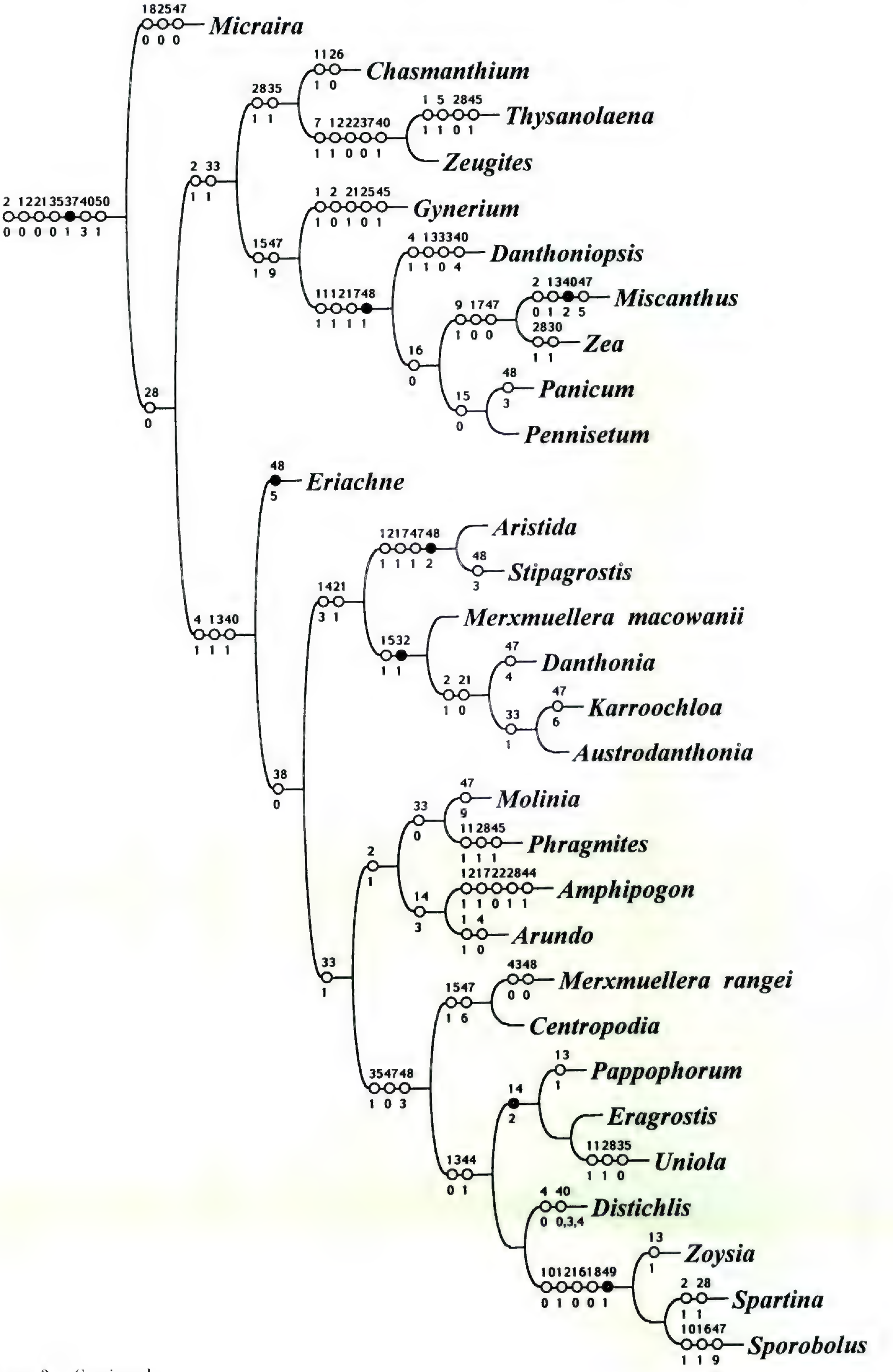


Figure 3. Continued.

(number of stigmas), 30 (highest order of stigmatic branching), 39 (lipid in the endosperm), 40 (endosperm starch grain syndrome), and 47 (base chromosome number).

For other structural characters, our assessment of character states is inadequate for use in phylogenetic analysis. For example, the woodiness of bamboo culms (char. 1) is due to numerous, closely spaced vascular bundles around the periphery of the culm, each bundle with massive sclerenchyma fiber caps on both sides, combined with heavily sclerified ground tissue, but isolated fiber strands may also occur (Soderstrom, 1981; Liese, 1998). It is not clear if the hardness of culms in *Arundo*, *Thysanolaena*, and *Gynerium* and a number of other "woody" taxa of the PACCAD Clade (Watson & Dallwitz, 1992) is derived in the same way; these taxa need more careful anatomical study. Similarly, the fringed membranous ligule of some Pooideae (char. 4) may not be the same as the ciliate membrane found in many members of the PACCAD Clade and requires developmental investigation. Spikelet pairing (char. 9) appears in various panicoid grasses and also in *Pharus*, but the pattern of development of the pairs in *Pharus* is unknown. Membranous lodicules (char. 21) are scored as being the same wherever they occur, but development of these structures has never been compared. Haustorial synergids are apparently uniquely derived within the Danthonioideae, but many taxa have not been investigated for this character. Embryo characters (35, 36, 37, 38) are often phylogenetically informative, but lack of observation of critical taxa makes their use difficult in some cases. Starch grains (char. 40) are classified according to their apparent structure when viewed with the light microscope. With much recent work done on the biochemistry and molecular genetics of starch granule formation (e.g., Whistler et al., 1984; Frazier et al., 1997), this character could and should be recircumscribed, although then much scoring will need to be redone. Arm cells and fusoid cells (chars. 45 and 46) are now seen to be ancestral in the family, yet their development and ultrastructure are poorly studied, and their physiological function is unknown. Comparisons of chromosome base numbers (char. 47) will almost certainly become more precise because of recent studies of nuclear genome arrangement (e.g., Gale & Devos, 1998).

Finally, some of the structural characters are genuine morphological puzzles, ones for which strict comparison is difficult. These include the floral bracts (glumes, lemmas, paleas, lodicules), which occur only in the grasses, and may not even occur in Anomochlooideae. Homology of the grass flower and spikelet affects characters 8, 18, and 19. C_4 photosynthesis (chars. 48 and 49) is known to be a set of characters that do not co-vary; its origin is still poorly understood. These characters are discussed in more detail below.

SPIKELETS AND FLOWERS

Spikelet. The flowers of most grasses are arranged in bracteate units known as spikelets. These usually consist of two bract-like appendages (glumes) at the base of a central axis (rachilla) on which are borne one or more florets, all in a distichous pattern. According to Clayton (1965: viii), the "grass spikelet never ceases to fascinate, for the simplicity of its theme is matched by the elegance of its variations." The apparent simplicity of the grass spikelet notwithstanding, its origin and homologies, as well as those of the grass flower, have been much debated (see reviews in Clifford, 1987, and Soreng & Davis, 1998).

The phylogeny highlights the difficulty of discussing the evolution of the grass spikelet. The standard spikelet (or some modification of it) is present in all members of the Pooideae and the PACCAD Clade (Figs. 4 and 5). The pattern of bracts and flowers, however, is variable among Pharoideae, Puelioideae, Bambusoideae, and Ehrhartoideae (Fig. 4). Pharoideae bear single flowers, each with a lemma, a palea, and a pair of glumes, but no rachilla extension (Fig. 4B; Judziewicz, 1987; Soderstrom et al., 1987). Puelioideae have multiflowered spikelets, in which each flower has a lemma and palea, and the whole unit has a pair of glumes (Fig. 4A). Proximal incomplete florets occur, but distal reduction is seen only in *Guaduella*. In Bambusoideae, the unisexual, one-flowered spikelets of Olyreae are standard, but the bracteate, rebranching spikelets (pseudospikelets) of many Bambuseae are difficult to interpret (Fig. 4F; Judziewicz et al., 1999). The multiflowered spikelet of *Streptogyna* presents no difficulties of interpretation, but in the Ehrhartoideae, extra proximal, sterile bracts (usually called sterile lemmas) are common (Fig. 4G), and extreme reduction of glumes is known (Oryzeae). It is not clear whether the proximal sterile bracts of Ehrhartoideae are truly homologous to the proximal incomplete florets that occur in Puelioideae and some bambusoids, or if they are phylogenetically (and possibly developmentally and genetically) distinct. The uncertain position of *Streptogyna* makes the homology assessment even more ambiguous.

Most characters of the spikelet and the floret (chars. 9–12 and 14–17) are treated as inapplicable

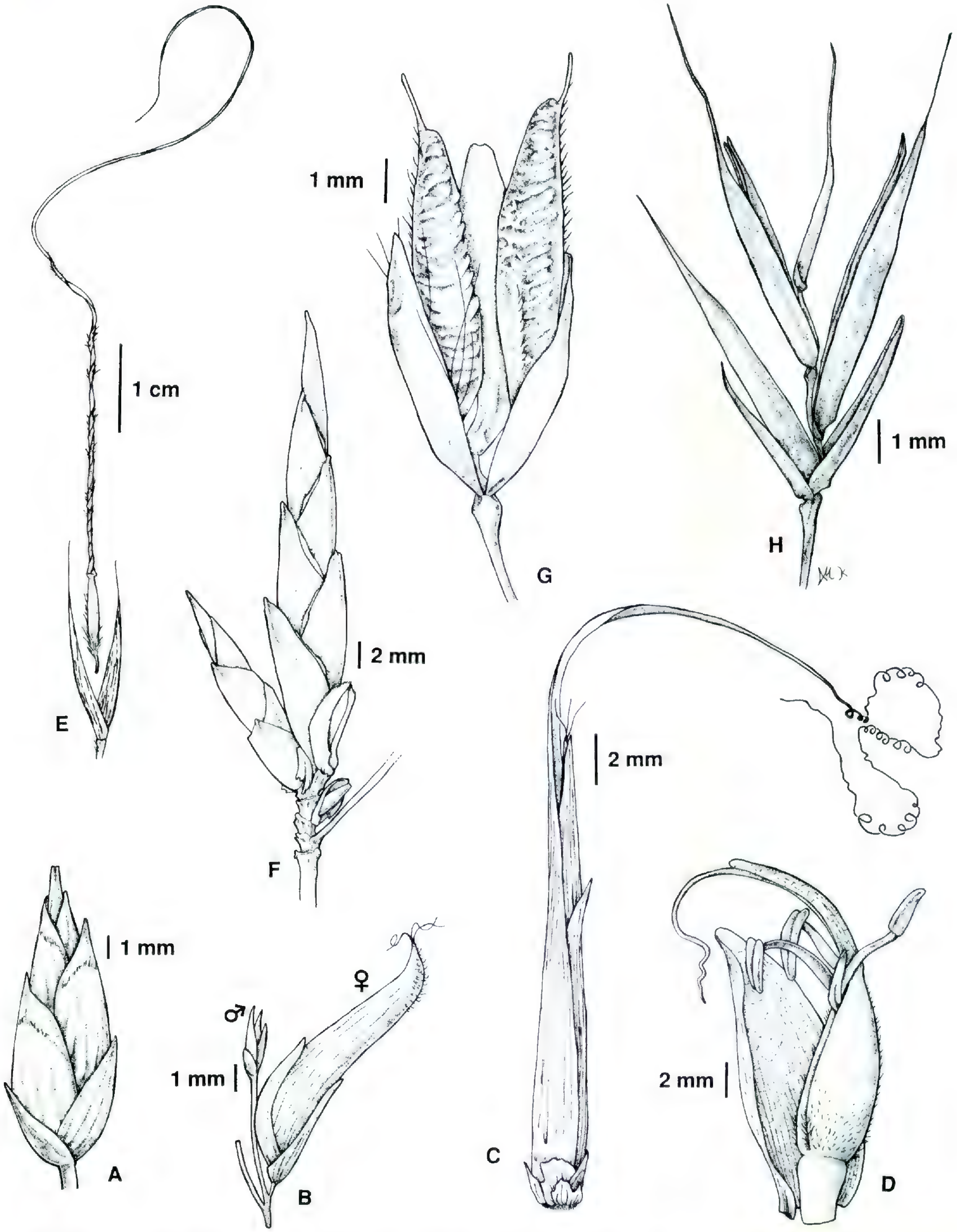


Figure 4. Spikelets and spikelet equivalents of early-diverging lineages and the BEP clade. —A. *Puelia schumanniana*, Puelioideae (*Lætouzey 12930*, US). —B. *Pharus mezii*, Pharoideae (*Hinton 16059*, US; redrawn from Judziewicz, 1987). —C. *Streptochaeta spicata*, Anomochlooideae (*Bailey & Bailey 723*, US; redrawn from Judziewicz & Soderstrom, 1989, from originals by A. Tangerini at US). —D. *Anomochloa marantoidea*, Anomochlooideae (*Calderón 2046*, US; redrawn from Judziewicz & Soderstrom, 1989, from originals by A. Tangerini at US). —E. *Stipa comata*, Pooideae (*Pearson s.n.*, ISC). —F. *Guadua chacoensis*, Bambusoideae (*Nee 35467*, ISC). —G. *Ehrharta bulbosa*, Ehrhartoideae (*Barker 1119*, ISC). —H. *Festuca idahoensis*, Pooideae (*Pohl 15642*, ISC).

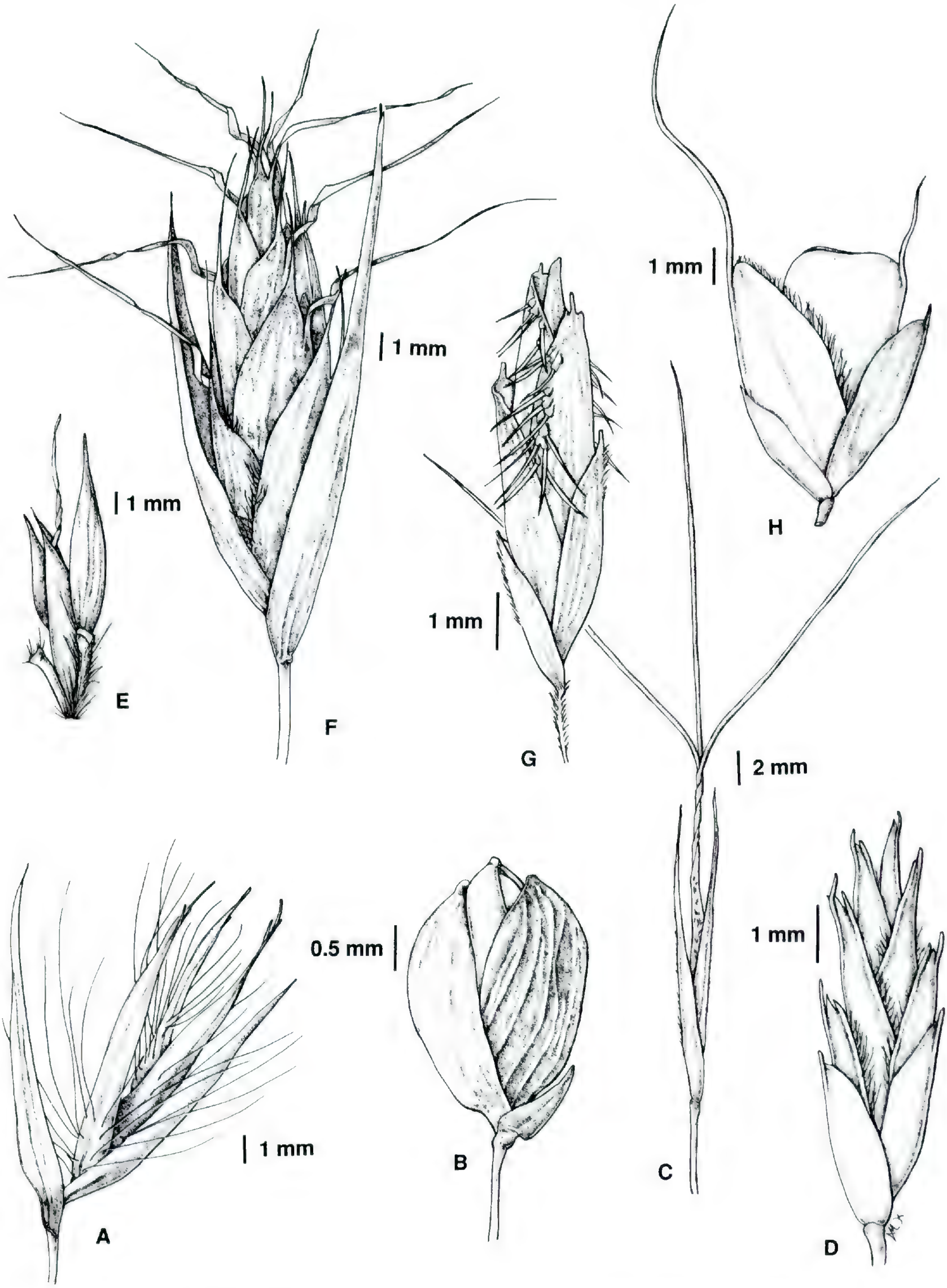


Figure 5. Spikelets of the PACCAD clade. —A. *Arundo donax*, Arundinoideae (*Bradley & Sears 3558*, ISC). —B. *Dichanthelium oligosanthes*, Panicoideae (*Lelong 2063*, ISC). —C. *Aristida arizonica*, Aristidoideae (*Griffiths 7373*, ISC). —D. *Tridens flavus*, Chloridoideae (*Thorne 18302*, ISC). —E. *Andropogon gerardii*, Panicoideae (*Clark s.n.*, teaching collection, ISC). —F. *Danthonia californica*, Danthonioideae (*Pohl 9459*, ISC). —G. *Centotheca lappacea*, Centothecoideae (*Liang 66250*, ISC). —H. *Chloris cucullata*, Chloridoideae (*Malacara & Gutierrez 33*, ISC).

when grass-type spikelets and florets are absent, as in the non-grass outgroups (see char. 8). Although pseudospikelets occur in some genera of Bambuseae, the two in the present study are regarded as having true spikelets and florets, and thus scorable for features of these structures.

Neither *Anomochloa* nor *Streptochaeta* (Fig. 4C and D) has structures clearly homologous with glumes, lemmas, or paleas, and thus neither can be described as having grass-type spikelets or florets. We therefore follow Clark and Judziewicz (1996) in using the term "spikelet equivalent" to refer to the flowering units of the inflorescences in the Anomochlooideae to emphasize this lack of recognizable homology. Characters of the spikelet and the floret are scored as inapplicable or ambiguous in these two genera except for char. 13 (see Appendix IV). The grass spikelet may have originated either before or after the divergence of Anomochlooideae. If before, then the spikelet was extensively modified in the long history of Anomochlooideae. The origin certainly must have occurred before divergence of Pharoideae. We refer to the clade of all grasses except Anomochlooideae as the Spikelet Clade.

Bracts outside of the spikelets subtend inflorescence axes and often have a blade. Short to elongate prophylls are usually present on the branches subtended by these bracts. Such bracts occur primarily in Bambuseae and Andropogoneae, but they are not necessarily homologous between the two groups (see Renvoize & Clayton, 1992). Well-developed subtending bracts are usually absent in other members of the Spikelet Clade, although there may be a ridge or scar which presumably represents the subtending bract at the base of the inflorescence branch.

Glumes. We have assumed here that glumes are homologous across the Spikelet Clade. Glumes are typically defined as the two sterile bracts at the base of the spikelet, but additional sterile bracts (usually called sterile lemmas or sterile florets even if there is no evidence of any, even vestigial, floral axis) may occur between the glumes and the flower-bearing lemmas (e.g., Ehrhartoideae, *Chusquea*, many Centothecoideae). In general, the first (lower) glume is abaxial and the second (upper) glume is adaxial (E. A. Kellogg, pers. obs.; Clifford, 1987), but the first glume may be adaxial in position, as in a number of Paniceae (Clifford, 1987). Grassl (1956) and Stapleton (1997) argued that the first glume is actually a prophyll for Andropogoneae and Bambuseae, respectively, and that the prophyll was displaced upward to assume the position and function of a glume. Even if the first glume is abaxial, the structure still could be derived as a prophyll if the axis rotated through 180° (Clifford, 1987). The gemmiparous bracts of many Bambuseae are essentially glumes with a bud in the axil. If the bud develops it becomes a second- or higher order pseudospikelet (Judziewicz et al., 1999). Glumes may be highly reduced or lost, as in Oryzeae. A glume-like prophyll at the base of the pseudospikelet is observed in many Bambuseae (Fig. 4F), although the axis bearing the prophyll is not elongated (McClure, 1966).

Numbers of florets. The rachilla may or may not extend beyond the most distal floret, and reduced or modified florets may be present below or above (or both below and above) the fertile ones. For this analysis, we have assumed that the grass flower is terminal to the axis on which it is borne. In the Anomochlooideae, there is no identifiable palea and thus the flower appears to be truly terminal to the main sympodial axes of the inflorescence in *Anomochloa*, as discussed in Soreng and Davis (1998); the same is true under Soderstrom's (1981) interpretation of the spikelet equivalent of *Streptochaeta*. Within the Spikelet Clade, however, the flowers are borne on lateral branches, as indicated by the presence of a prophyll (i.e., the palea) in the proximal, adaxial position on the branch. This pattern is clear in those taxa with multiflowered spikelets or spikelets with one floret and a rachilla extension. There are a number of taxa with a single floret and no rachilla extension, including the Pharoideae, in which a well-developed palea is found in the floret. It is simple enough to imagine the reduction of the branch apex to the point where no evidence of a rachilla extension can be observed, but, as Soreng and Davis (1998) noted, the presence of a single-flowered floret (or equivalent) appears to be plesiomorphic for the family. This implies that either the rachilla extension and additional fertile florets evolved subsequently, or that multiflowered spikelets evolved before the divergence of the Pharoideae, and reduction to a single fertile floret occurred in that lineage (Soreng & Davis, 1998). Clearly, single-flowered spikelets evolved a number of times in various lineages in the BEP + PACCAD Clade.

Lemma. Each floral axis is subtended by a lemma (Fig. 6A and B), a structure that appears to be universally present across the Spikelet Clade. The lemma apparently is formed wholly by the spikelet meristem, and thus is a bract on the rachilla (Clifford, 1987). Lemma morphology is extremely variable, but the number of nerves is consistently odd, varying from 1 to 15 (Clifford, 1987). Some taxa

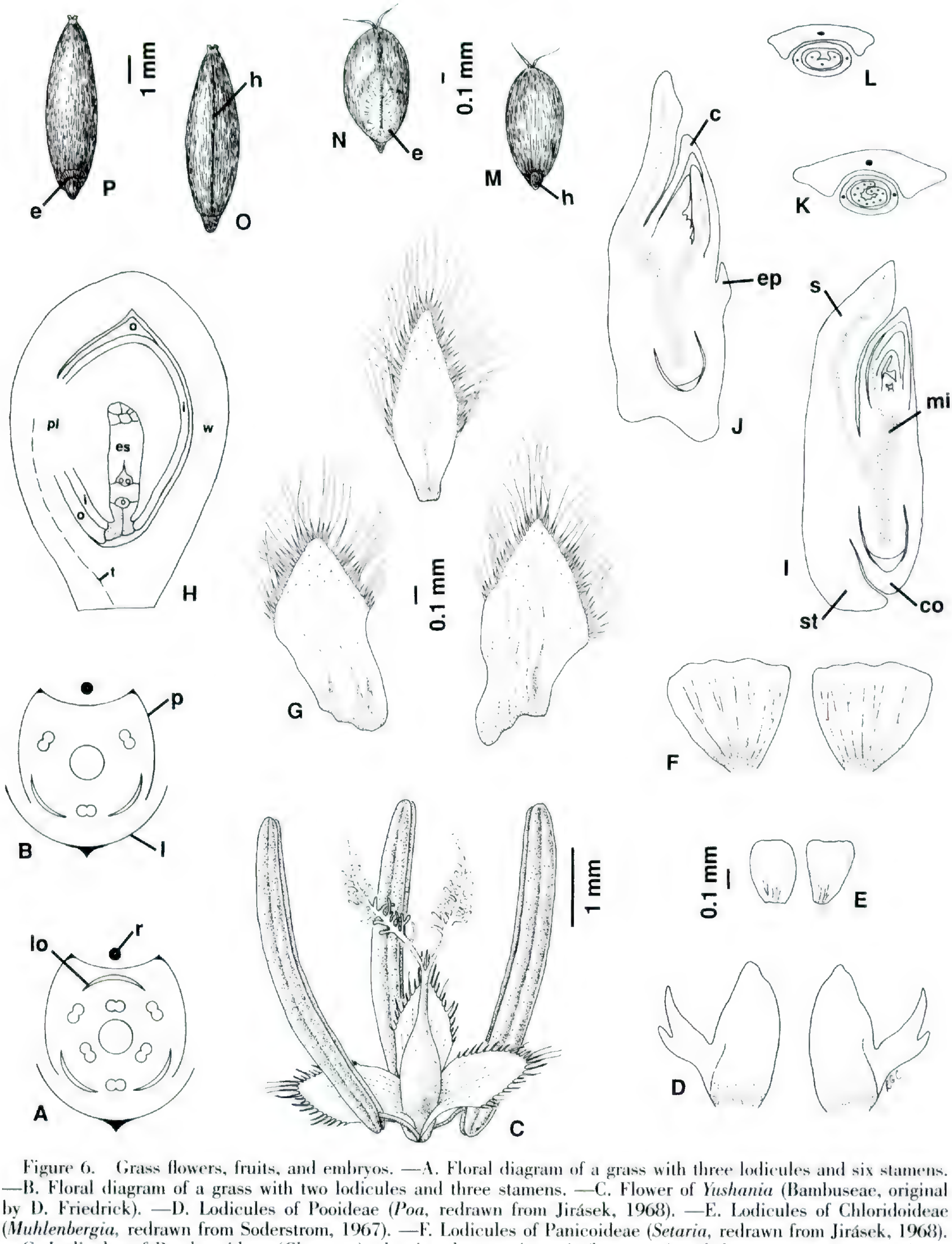


Figure 6. Grass flowers, fruits, and embryos. —A. Floral diagram of a grass with three lodicules and six stamens. —B. Floral diagram of a grass with two lodicules and three stamens. —C. Flower of *Yushania* (Bambuseae, original by D. Friedrick). —D. Lodicules of Pooideae (*Poa*, redrawn from Jirásek, 1968). —E. Lodicules of Chloridoideae (*Muhlenbergia*, redrawn from Soderstrom, 1967). —F. Lodicules of Panicoideae (*Setaria*, redrawn from Jirásek, 1968). —G. Lodicules of Bambusoideae (*Chusquea*), showing the anterior pair (lower two) and the posterior one (upper). —H. Generalized ovule section (Danthonioideae) showing haustorial synergids (stippled) (redrawn from Verboom et al., 1994). —I. Longitudinal section of a panicoid embryo showing presence of a scutellar tail (st) and the elongated mesocotyl internode (mi). —J. Longitudinal section of a pooid embryo showing presence of an epiblast (ep). —K. Cross section of a panicoid embryo apex showing overlapping embryonic leaf margins. —L. Cross section of a pooid embryo apex showing embryonic leaf margins that meet. M, N. Caryopsis of *Eustachys* (Chloridoideae). —M. Hilum side, showing a punctiform hilum (h). —N. Embryo side, showing the large embryo (e). O, P. Caryopsis of *Chusquea* (Bambusoideae) (redrawn from McClure, 1973). —O. Hilum side, showing a linear hilum (h). —P. Embryo side, showing the small embryo (e). c—coleoptile; co—coleorhiza; e—embryo; ep—epiblast; es—embryo sac; h—hilum; i—inner integument; l—lemma; lo—lodicule; mi—mesocotyl internode; o—outer integment; p—palea; pl—placenta; r—rachilla; s—scutellum; st—scutellar tail; t—vascular trace to placenta; w—ovary wall.

have more than two glumes at the base of the spikelet (see Glumes), but the sterile lemma of the panicoid spikelet does appear to represent an evolutionary reduction from a fertile floret.

Palea. The palea (Fig. 6A and B) is widely interpreted as a prophyll (Linder, 1987; Stapleton, 1997; Clayton, 1990; Soreng & Davis, 1998; Judziewicz et al., 1999), and prophylls do occur in the inflorescences of many Bambuseae and Andropogoneae (Judziewicz et al., 1999; E. A. Kellogg, unpublished obs.) relative to subtending bracts in precisely the same way a prophyll is related to a subtending leaf. Although the "palea as prophyll" explanation may be more parsimonious, the derivation of the palea from the fusion of two sepals also has been supported (Schuster, 1910; Stebbins, 1974; Irish, 1998; Schmidt & Ambrose, 1998). Nuñez (1968) and Clifford (1987) have suggested that the odd-nerved, often 1-keeled paleas in the Oryzeae represent a separate origin of a palea-like structure, but this interpretation is inconsistent with the phylogeny. There is no reason to suppose paleas have been replaced with something different in Oryzeae. Single-flowered spikelets lacking a palea (e.g., *Alopecurus*) probably represent loss of the rachilla extension and the palea on the floral axis.

Vegetative branching in the grasses and other monocots typically involves a leaf with a bud or branch in its axil, although bud displacement is observed in some palms and grasses (Dahlgren et al., 1985; Serebryakova, 1971; Fisher & Dransfield, 1977). The first appendage of the branching axis is an adaxial two-keeled bract (= prophyll). This prophyll encloses the bud, but often persists once the branch develops. The origin of the vegetative prophyll is not clear, but whether it is a single structure or the result of fusion of two bracts, ultimately it is foliar in nature (Stebbins, 1974). The relationship between the subtending leaf and the prophyll is generally constant, and therefore a prophyll marks the presence of a lateral branch. If the vegetative branching pattern is reiterated in the inflorescence, then the palea ought to be homologous to a prophyll, and prophylls should occur in fully bracteate inflorescences as the adaxial first appendage of branches in the axils of subtending bracts.

Flower. Grass flowers are made up of a gynoecium, androecium, and two or three flap-like structures (lodicules) that force the floret open at maturity (Fig. 6A–G). The literature on the anatomy and development of the grass flower should probably be reinterpreted in the light of the present phylogeny. The origin and homology of the lodicules continue to be controversial. The grass flower has been interpreted variously as dichlamydeous (with two perianth whorls, the palea representing two fused sepals and the lodicules the petals), monochlamydeous (with only one perianth whorl, the lodicules, present, and the palea homologous to a prophyll), achlamydeous (with no perianth, the lodicules representing modified bracts or stipules), or pseudanthial (with the flower representing a highly reduced branch system, and the lodicules derived from leaves or branches) (Clifford, 1987). The monochlamydeous interpretation of the grass flower is the most widely accepted. Recent molecular genetic studies provide support for a petaloid homology of the lodicules (Irish, 1998; Schmidt & Ambrose, 1998; Ambrose et al., 2000), thus rejecting the achlamydeous and pseudanthial hypotheses. Ambrose et al. (2000) also provided genetic evidence that the palea and possibly also the lemma have characteristics in common with an outer perianth whorl, thus suggesting that the dichlamydeous interpretation might be revived. Bossinger (1990) and Pozzi et al. (2000) described mutations in barley in which the lemma is converted to a leaf. This may suggest that the lemma is more leaf-like than sepal-like. Kellogg (2000a) suggested that these two interpretations are not mutually exclusive, and that the complex structure of the grass spikelet may reflect simultaneous expression of both leaf and floral developmental "programs." If the latter interpretation is correct, then it may be meaningless to discuss whether the lemma is "really" a leaf or a sepal.

Androecium. All six stamens (arranged in two alternating whorls of three stamens each, Fig. 6A; see Clifford, 1987) are plesiomorphically present within the study group and at the point of origin of the grass family, but the entire outer whorl is lost in the Restionaceae. Within the grass family, all six stamens (both whorls) are maintained in the three earliest-diverging subfamilies (except for the loss of the inner anterior pair in *Anomochloa*, as noted above). The outer whorl is maintained throughout most of the grass family, except for the various autapomorphic losses and polymorphisms noted above. Loss of the entire inner whorl (e.g., Fig. 6B) is interpreted as a synapomorphy of the BEP + PACCAD Clade, but in one subclade within this large group (the bambusoid/ehrhartoid alliance) there are three or more independent reversions to presence of this whorl, and possibly a secondary loss in *Leersia*. An alternative interpretation of the outer stamen whorl, in the context of the present reconstruction of phylogenetic relationships, and involving no secondary origins, would have the in-

ner whorl lost independently in Pooideae and the PACCAD Clade, as well as in a series of small lineages within the bambusoid/ehrhartoid alliance. Alternatively, if a PACCAD + Pooideae lineage is considered, loss of the inner stamen whorl could be interpreted as a synapomorphy of that clade; the presence of this whorl in some genera of Ehrhartoideae and Bambusoideae might then be interpreted as retention of a plesiomorphy, while the absence in others would be interpreted as having arisen independently of the loss in the PACCAD + Pooideae.

BIOCHEMISTRY

The phylogeny suggests that the C_4 photosynthetic pathway has evolved multiple times within the PACCAD Clade. In the C_4 pathway, the Calvin-Benson cycle, and hence Rubisco, is relegated to the bundle sheath cells surrounding the veins (Hattersley & Watson, 1992; Sinha & Kellogg, 1996). Phosphoenolpyruvate carboxylase then catalyzes CO_2 reduction in the mesophyll to produce the four-carbon compound oxaloacetate. This compound is then reduced to malate or aspartate and transported to the bundle sheath, where the newly fixed CO_2 is immediately removed and taken up by Rubisco. This keeps CO_2 concentration high at the active site of Rubisco, preventing competition by O_2. Consistent with the constant flow of materials between the bundle sheath and mesophyll, C_4 species (Fig. 7C) have closer vein spacing than C_3 species (Fig. 7B). Other anatomical manifestations of C_4 photosynthesis include enlarged bundle sheath cells (Kranz anatomy), closely packed chlorenchyma cells, and in some, radiate chlorenchyma (Fig. 7C).

Despite the foregoing generalizations, C_4 photosynthesis actually represents a suite of characters, rather than a single genetic and phylogenetic change. Only down-regulation of Rubisco in the mesophyll, up-regulation of PEP carboxylase, and closer vein spacing are common to all C_4 grass lineages. The four-carbon compound that transports the carbon to the bundle sheath may be malate or aspartate, the decarboxylating enzyme may be a malic enzyme using NAD as a co-factor (NAD-ME), or using NADP (NADP-ME). If the former, additional decarboxylation activity may be provided by PEP carboxykinase, so PCK activity is dependent upon the presence of NAD-ME (Kanai & Edwards, 1999). Some C_4 grasses have only one bundle sheath, whereas others have two. In those with two bundle sheaths the inner sheath may be made up of thick-walled cells, forming a conventional mestome sheath, or it may be parenchymatous. There are additional differences in expression of the enzymes involved in photosynthesis (Sinha & Kellogg, 1996). For example, all Chloridoideae except for the C_3 *Eragrostis walteri* and *Merxmuellera rangei* form aspartate, use the NAD-malic enzyme, and have double bundle sheaths, with the inner one of thick-walled cells. (*Centropodia* has not been biochemically typed, but is anatomically the same as other NAD-ME taxa.) *Stipagrostis*, in Aristidoideae, is similar to the chloridoids in having two bundle sheaths, the outer of which appears to be carbon-reducing (Sinha & Kellogg, 1996). *Aristida* itself forms malate, using the NADP-malic enzyme; unlike other NADP taxa, it has two bundle sheaths. The ultrastructure of the outer sheath is similar to that of NADP species, but the inner sheath is more like an NAD or PCK plant; the extent to which these sheaths are developed varies throughout the genus (Brown, 1977; Carolin et al., 1973). *Eriachne* also uses NADP-ME and has a double bundle sheath, but unlike *Aristida* the inner sheath has thick-walled cells. The C_4 Panicoideae also vary biochemically and histologically, although large groups are uniform. For example, the entire tribe Andropogoneae (ca. 100 genera and 1000 species) uses NADP-ME and has a single bundle sheath.

Both the phylogeny and structural/biochemical data indicate that the C_4 pathway is not homologous wherever it occurs. The close relationship of the C_4 lineages, however, suggests that there were a set of changes at the base of the entire PACCAD Clade that made the pathway easier to evolve. If this were true, then those changes, whatever they were, would be homologous, even though the final manifestations of the pathway are not.

IMPLICATIONS FOR MORPHOLOGICAL EVOLUTION

The phylogeny provides a unique and powerful tool for description of evolutionary pattern (Kellogg, 2000a). Major clades and evolutionary transitions are summarized in Figure 8. Additional detail can be found at http://www.virtualherbarium.org/grass/gpwg/default.htm.

Sister relationship of Poaceae and Joinvilleaceae. The presence of long and short cells in the leaf epidermis (char. 42), with at least some of the short cells containing silica bodies, unambiguously supports the sister relationship of Joinvilleaceae and Poaceae. This arrangement is apparently unique among angiosperms (Campbell & Kellogg, 1987; Kellogg & Linder, 1995). The presence of the 6.4 kb inversion in the chloroplast genome (char. 51) is also an unambiguous synapomorphy supporting this sister relationship, although it is not

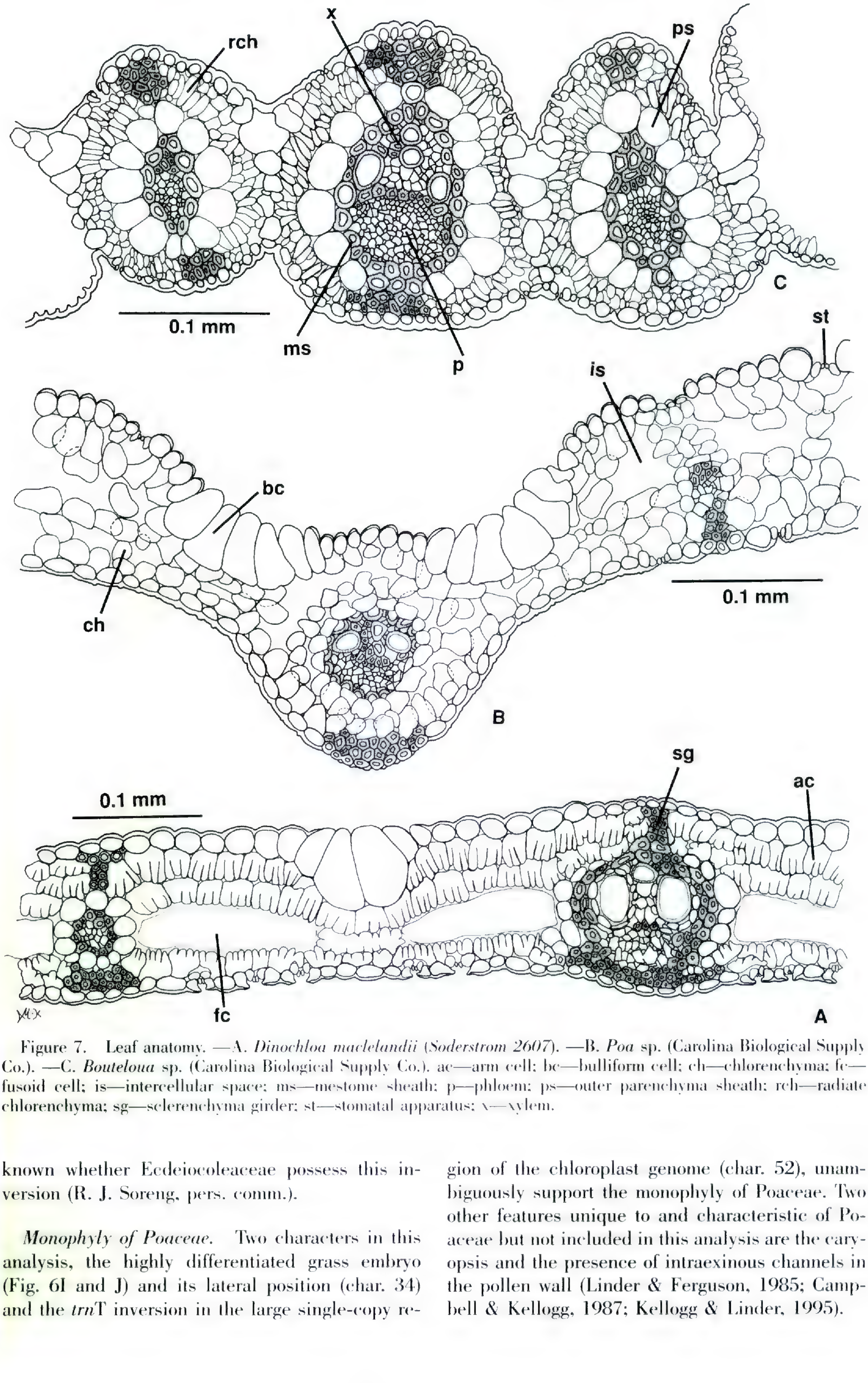
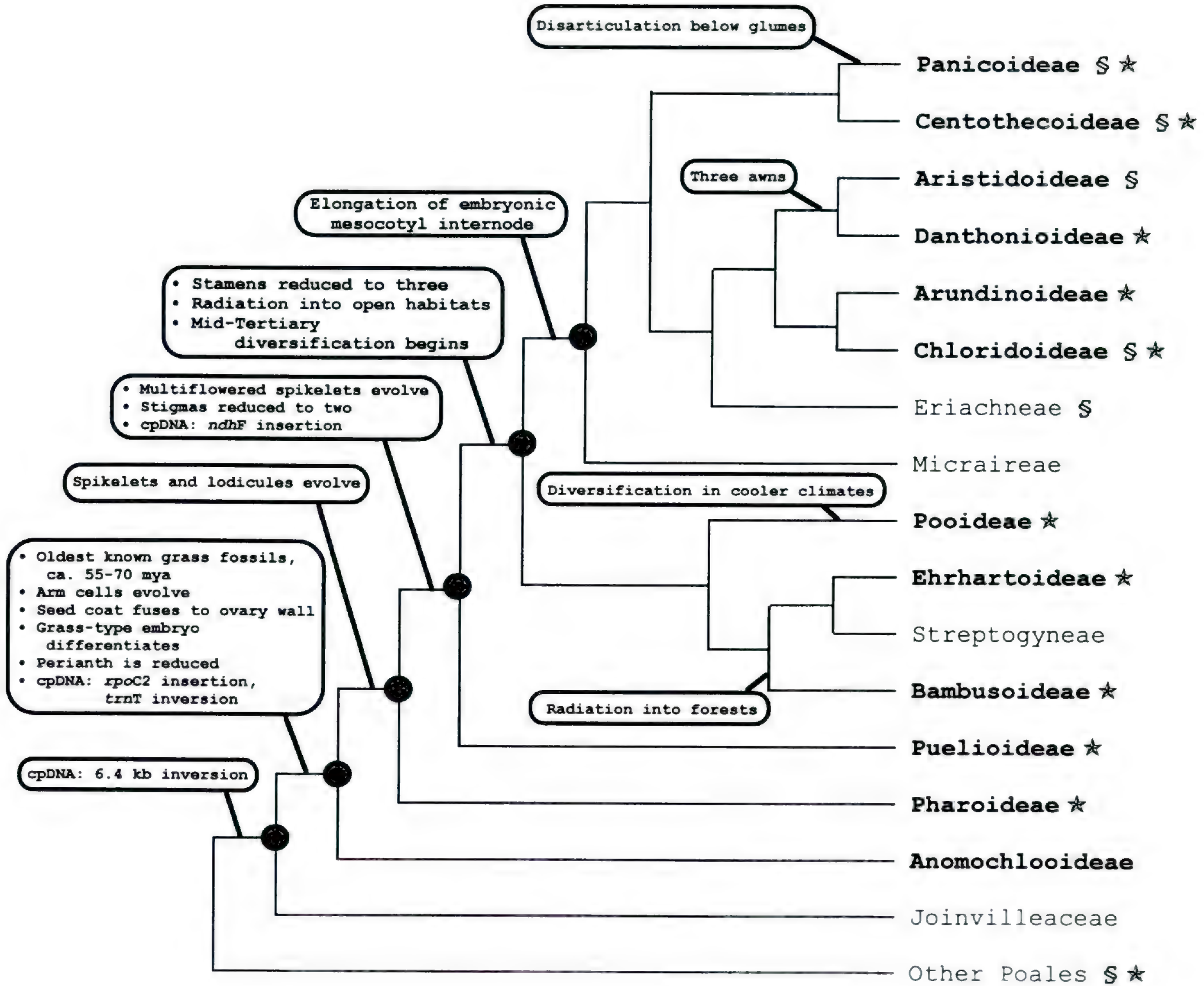


Figure 7. Leaf anatomy. —A. *Dinochloa maclelandii* (*Soderstrom 2607*). —B. *Poa* sp. (Carolina Biological Supply Co.). —C. *Bouteloua* sp. (Carolina Biological Supply Co.). ac—arm cell; bc—bulliform cell; ch—chlorenchyma; fc—fusoid cell; is—intercellular space; ms—mestome sheath; p—phloem; ps—outer parenchyma sheath; rch—radiate chlorenchyma; sg—sclerenchyma girder; st—stomatal apparatus; x—xylem.

known whether Ecdeiocoleaceae possess this inversion (R. J. Soreng, pers. comm.).

Monophyly of Poaceae. Two characters in this analysis, the highly differentiated grass embryo (Fig. 6I and J) and its lateral position (char. 34) and the *trn*T inversion in the large single-copy region of the chloroplast genome (char. 52), unambiguously support the monophyly of Poaceae. Two other features unique to and characteristic of Poaceae but not included in this analysis are the caryopsis and the presence of intraexinous channels in the pollen wall (Linder & Ferguson, 1985; Campbell & Kellogg, 1987; Kellogg & Linder, 1995).


Disarticulation below glumes
Panicoideae § ★
Centothecoideae § ★
Three awns
Aristidoideae §
Danthonioideae ★
Elongation of embryonic mesocotyl internode
• Stamens reduced to three
• Radiation into open habitats
• Mid-Tertiary diversification begins
Arundinoideae ★
Chloridoideae § ★
Eriachneae §
• Multiflowered spikelets evolve
• Stigmas reduced to two
• cpDNA: ndhF insertion
Micraireae
Spikelets and lodicules evolve
Diversification in cooler climates
Pooideae ★
• Oldest known grass fossils, ca. 55-70 mya
• Arm cells evolve
• Seed coat fuses to ovary wall
• Grass-type embryo differentiates
• Perianth is reduced
• cpDNA: rpoC2 insertion, trnT inversion
Ehrhartoideae ★
Streptogyneae
Radiation into forests
Bambusoideae ★
Puelioideae ★
cpDNA: 6.4 kb inversion
Pharoideae ★
Anomochlooideae
Joinvilleaceae
Other Poales § ★

The caryopsis is a single-seeded, usually dry, indehiscent fruit with the pericarp fused to the seed coat in the hilar region and otherwise closely adnate (Sendulsky et al., 1987). The caryopsis develops from a unilocular ovary containing a single ovule (Fig. 6H). Within Poaceae, the basic caryopsis has been modified to the fleshy (baccoid) or the achene-like (nucoid) caryopses of some woody bamboos (Sendulsky et al., 1987) or the follicoid or cistoid caryopses of some chloridoids in which the seed is free from the pericarp or separates from it when moistened.

The highly differentiated grass embryo and its lateral position at the base of the caryopsis (char. 34; Fig. 6I and J) are synapomorphies for the family (Campbell & Kellogg, 1987; Kellogg & Linder, 1995). In the grasses, the embryo has leaves, vascular tissue, and clearly localized shoot and root meristems before the fruit is dispersed, and thus looks much more like a seedling than the embryos of non-grass relatives (Fig. 6I and J; Reeder, 1957; Sendulsky et al., 1987). Constant features of the grass embryo are the scutellum, coleoptile, and coleorrhiza. A scutellar cleft (Fig. 6I) may or may not separate the scutellum from the coleorrhiza, and the epiblast, an extra flap of tissue opposite the scutellum, may be present (Fig. 6J) or absent (Fig. 6I). Whether or not the embryonic leaf margins meet or overlap varies throughout the family (Fig. 6K and L).

Changes in the inflorescence occurred between the time that *Joinvillea* diverged and the time of divergence of Anomochlooideae. Relative to most of their poalean sister families (excluding Restionaceae, Ecdeiocoleaceae, and Centrolepidaceae), the grasses, including Anomochlooideae, have well-developed bracts (what are normally called subtending bracts, prophylls, glumes, lemmas, and paleas) subtending and enclosing contracted inflorescence branches and flowers (Figs. 4 and 5). This appears to be a derived character marking the origin of the family, although this was not used explicitly as a character in this analysis. Under this interpretation, the characteristic grass spikelet (found in all grasses except Anomochlooideae) is then likely the result of a cumulative series of changes that occurred during the early history of the family. Clayton (1990), however, pointed out that there are well-developed bracts in the spikelets of Restionaceae, and what could be interpreted as subtending bracts are present, although not necessarily well developed, in Joinvilleaceae (Dahlgren et al., 1985). Clayton (1990) also noted that if the palea in grasses is interpreted as a prophyll, then there is no homolog for it among other Poales; however, prophylls are occasionally found at the base of spikelets in Restionaceae (H. P. Linder, pers. obs.).

The results of this analysis and prior studies (Clark et al., 1995; Soreng & Davis, 1998) support the following as plesiomorphic within the grass family: an herbaceous, perennial, rhizomatous habit; pseudopetiolate and relatively broad leaf blades bearing multicellular microhairs and anatomically with commissural veins, fusoid cells (Fig. 7A), and alternating long and short cells on the epidermis with at least some of the short cells including silica bodies; leaves with an adaxial ligule and open sheaths; a highly bracteate inflorescence; one-flowered spikelets or spikelet equivalents; six stamens in two whorls with tetrasporangiate, dithecal anthers; monoporate pollen with intraexinous channels in the wall; a uniloculate, uniovulate gynoecium with three stigmas and one order of stigmatic branching; a basic caryopsis with a linear hilum; a highly differentiated, laterally positioned embryo with a scutellum, coleoptile, coleorhiza, and a negligible mesocotyl internode; *Festuca*-type starch grains in the endosperm; and the C_3 photosynthetic pathway. The grass spikelet and lodicules may have evolved before the divergence of the Anomochlooideae and the rest of the family, and were lost in the Anomochlooideae (cf. Soreng & Davis, 1998), but these features are not plesiomorphic under the present optimization. Bisexual flowers are probably also plesiomorphic, but unisexuality evolved early and in a number of different lineages within the family.

←

Figure 8. Summary phylogeny of the grasses indicating significant morphological, ecological, and molecular (cpDNA = chloroplast DNA) events in the evolution of the family. Infrequent losses, parallel gains, and reversals are not shown for these characters. The 12 subfamilies recognized by the GPWG appear in boldface. Poales sensu APG include Cyperaceae. Marked taxa: (star) At least some included species have unisexual flowers/florets; (§) At least some included species have a C_4 carbon fixation pathway, Kranz anatomy, or both. Dark circles indicate nodes strongly supported by all data combined (bootstrap > 99; Bremer support > 16). Subfamilies with common names: Aristidoideae (wiregrasses, etc.), Arundinoideae (reeds, etc.), Bambusoideae (bamboos), Chloridoideae (lovegrasses, tef, etc.), Danthonioideae (oatgrasses, pampas grass, etc.), Ehrhartoideae (rice, wild-rice, etc.), Panicoideae (maize, panic grasses, millets, sorghum, sugar cane, etc.), and Pooideae (barley, brome grasses, oats, rye, wheat, etc.).

Anomochlooideae. The basal divergence between Anomochlooideae and the rest of the Poaceae (the Spikelet Clade) is well supported based on molecular evidence. Monophyly of Anomochlooideae, however, is supported morphologically only by the unreversed presence of the adaxial ligule as a fringe of hairs, a character that appears elsewhere in the family. The pulvinus at the summit of the pseudopetiole is a possible synapomorphy for this clade but requires further study to determine similarities with the structure in other grasses. Molecular support for this clade may be due at least in part to long-branch attraction (see Unresolved questions).

Anomochlooideae have been recognized as a separate family (Nakai, 1943), a point of view that is completely consistent with the phylogeny. We have chosen here to retain Anomochlooideae within Poaceae because of the strong synapomorphies linking them (notably the caryopsis and the highly differentiated embryo, see Fig. 8). Retention of Anomochlooideae in Poaceae is also taxonomically conservative, in line with all previous studies of the family, and consistent with the efforts of the APG to limit monotypic or small families (Chase et al., 2000a, b).

Anomochlooideae are variable with respect to embryonic leaf margins and the epiblast. Embryonic leaf margins meet and the epiblast is present in *Anomochloa*, whereas the embryonic leaf margins overlap and the epiblast is absent in *Streptochaeta* (Judziewicz & Soderstrom, 1989). Both genera have an inconspicuous scutellar cleft. The coleoptile is usually represented as a more or less conical "cap" protecting the embryonic leaves and shoot apex, but the coleoptile margins are entirely free and overlapping in *Streptochaeta*, whereas in *Anomochloa* the margins at the base of the coleoptile are fused but free toward the apex, as is also seen in Pharoideae (Reeder, 1953; Judziewicz & Soderstrom, 1989).

The inflorescences of both *Anomochloa* and *Streptochaeta* are bracteate, but the lack of clear homology of these bracts with those of the standard grass spikelet has been noted. The spikelet equivalents in both *Anomochloa* and *Streptochaeta* are one-flowered and bisexual. The upper bract in *Anomochloa* exhibits a laminar anatomical structure, with the transversely elongated cell layer subjacent to the abaxial epidermis of the bract (Judziewicz & Soderstrom, 1989). This laminar structure is not found in bracts of *Streptochaeta*. (Similar but not identical laminar anatomy characterizes female lemmas in Pharoideae.) As Soreng and Davis (1998) pointed out, if the upper bract of the spikelet equivalent of *Anomochloa* is indeed homologous to a standard grass lemma, a palea is lacking and the flower is terminal, whereas in a true grass floret the flower is borne on a lateral axis as indicated by the presence of the palea if it is interpreted as a prophyll. Some authors interpret the distal three bracts of the spikelet equivalent of *Streptochaeta* as lodicules and the next proximal two bracts as a bifid palea (e.g., Clayton, 1990), but there is no compelling evidence for this. The spikelet equivalent of *Streptochaeta* might represent a condensed branching system (Soderstrom, 1981), but it is not a pseudospikelet as found in the Bambuseae. In any case, lack of a palea in *Streptochaeta* also implies that the flower is terminal.

The Spikelet Clade (Pharoideae + [Puelioideae + {BEP + PACCAD}]). This clade, which includes all of the grasses except for Anomochlooideae, is defined by the unambiguous presence of true grass spikelets, florets (char. 8), and lodicules (char. 18). The plesiomorphic condition of the spikelet is clearly the presence of a pedicel (char. 10), two glumes, and a well-developed lemma and palea in the floret. The single-flowered spikelet may be synapomorphic for the family above the point of divergence of Anomochlooideae, with a transformation to multiflowered spikelets above Pharoideae and then numerous reversals, but the first true spikelets in grasses may have been multiflowered (see discussion under Spikelet). The plesiomorphic condition for lodicules is clearly three (char. 19), unfused (char. 20), and with a distally membranous portion (char. 21). Presence of unisexual flowers may be synapomorphic for this clade, with numerous reversals to bisexual florets, but it may be more likely that unisexuality arose multiple times. A base chromosome number of $x = 12$ (char. 47) was established before the divergence of Pharoideae.

Pharoideae. Monophyly of this clade is strongly supported by the presence of resupinate leaf blades, oblique lateral veins in the leaf blades, and uncinate hairs wholly or partially covering the female lemmas (Judziewicz, 1987). The female lemmas exhibit a laminar anatomical structure similar to that of Anomochlooideae, but the transversely elongated cell layer is subjacent to the adaxial epidermis. In Pharoideae, Anomochlooideae, and some Bambusoideae (Ghopal & Ram, 1985), the coleoptile margins are free for at least a portion of their length, but the distribution of this feature in the rest of the BEP + PACCAD Clade is not well documented. Pharoideae embryos have an epiblast and a small scutellar cleft, and embryonic leaf margins meet (Judziewicz, 1987). The Pharoideae uni-

formly exhibit one-flowered, unisexual, paired (char. 9) spikelets (Fig. 4B). When present in the male spikelets, the three lodicules are small, which may be plesiomorphic or may represent a reduction. Disarticulation is variable, in that the whole inflorescence may disarticulate as in *Scrotochloa*, or the whole inflorescence or branches usually disarticulate in *Pharus*, or female spikelets disarticulate above the glumes in *Leptaspis*, and perhaps also in the other genera (Soderstrom et al., 1987). These, along with the uncinate hairs, appear to be adaptations to epizoochorous dispersal. Multicellular microhairs (char. 43) are lost in Pharoideae.

The Bistigmatic Clade (Puelioideae + [BEP + PACCAD]). This clade is marked by three morphological synapomorphies: transformation from three to two stigmas (char. 29), transformation from one to two orders of stigmatic branching (char. 30), and presence of the 15 bp *ndh*F insertion (char. 53). Multiple florets per spikelet (char. 12; Fig. 5) may have arisen within Puelioideae as shown in Figure 3, or in the common ancestor of the Puelioideae + (BEP + PACCAD) clade. Regardless of which scenario is correct, reversals to one floret occurred numerous times in the BEP + PACCAD Clade. Disarticulation above the glumes (char. 16) clearly is established before the divergence of Puelioideae.

Puelioideae. The forest habitat and broad, pseudopetiolate leaf blades of the Anomochlooideae and Pharoideae are retained in this subfamily, but no unique morphological synapomorphies for Puelioideae have been identified. The culms apparently do not produce aerial branches, nor basal tillering as in Anomochlooideae and Pharoideae. The presence of proximal female-sterile florets in the spikelet (char. 11) is an unreversed synapomorphy for Puelioideae in this analysis, but is also a synapomorphy for Paniceae and autapomorphic for multiple other taxa on the tree. In Puelioideae, the pattern of sexuality in the spikelets is somewhat more complex than in many other subfamilies, but within a spikelet, at least some proximal florets are male. In *Guaduella*, the 1 to 3 proximal florets are male, and additional florets are bisexual with the distalmost one or few reduced, but in *Puelia* the proximal 3 to 6 florets are male or neuter, with the single apical floret unisexual and female. Multicellular microhairs (char. 43) are lost in *Puelia*, and a reversion to three stigmas (char. 29) occurs in some species of *Puelia*.

The BEP + PACCAD Clade. This clade includes the vast majority of grass species. Six morphological synapomorphies support its monophyly: loss of the pseudopetiole (char. 7), reduction to two lodicules (char. 19), loss of the inner whorl of stamens (chars. 23 and 24), and loss of arm and fusoid cells (chars. 45 and 46). The pseudopetiole is regained in Bambusoideae, as well as in a few members of the PACCAD Clade. Arm and fusoid cells are also regained in Bambusoideae. The inner whorl of stamens is interpreted as having been regained three or four times within the bambusoid/ehrhartoid clade. Within the BEP + PACCAD Clade, the lamina on the first seedling leaf is lost only in Bambusoideae and Oryzeae. Unisexual florets have evolved in most lineages of this clade, e.g., Olyreae (Bambusoideae), *Zizania* (Ehrhartoideae), *Lamarckia* (Pooideae), several genera of Chloridoideae and Centothecoideae, and very commonly in Panicoideae. Most lineages include taxa with one floret per spikelet and taxa with multiple florets per spikelet. The presence or absence of an epiblast is variable, as is the presence or absence of the scutellar cleft, although in the PACCAD Clade the scutellar cleft is generally present (Reeder, 1957).

The large number of reversals hypothesized in this part of the tree raises a number of intriguing questions regarding morphological evolution. We do not know anything about the development or underlying genetics of the characters, so we are forced into the agnostic assumptions that gains and losses are equally likely, and that pseudopetioles, arm cells, fusoid cells, epiblasts, and unisexual flowers are all developmentally and genetically the same wherever they occur. The changes that we interpret as reversals could actually represent retained primitive characters if loss of these characters is more likely than their regain. Equally possible, the changes interpreted as reversals could represent the origin of novel characters that look superficially similar to ancient ones. We have some evidence for the latter (see below) in that arm cells in the bambusoids are actually morphologically different from those in Anomochlooideae and Pharoideae (Zhang & Clark, 2000). Finally, the character optimizations reflect the hypothesis that the BEP Clade is monophyletic. If, as we outline below, the Pooideae are actually sister to the PACCAD Clade—a hypothesis that is neither favored nor excluded by the data—then the pattern of morphological evolution is different.

The BEP Clade (= BOP clade of Clark et al., 1995). This clade is supported by molecular sequence data, particularly from *ndh*F, *rpo*C2, and *phy*B (see Results), but other data sets support a

Pooideae + PACCAD clade (Soreng & Davis, 1998). In this analysis, constraining Pooideae + PACCAD as monophyletic was only slightly less parsimonious than BEP + PACCAD (see Results). In addition, no morphological synapomorphies supporting the BEP Clade have been identified. Loss of the lemma awn is optimized to this node, but awns are regained in many taxa within the BEP Clade. The lack of sequence data for *Streptogyna* contributes to the uncertainty of its position within the BEP Clade and may also affect assessment of the monophyly of the clade. *Streptogyna* appears as sister to Ehrhartoideae (Fig. 1), but in other analyses of these data it appears as sister to the rest of the BEP Clade.

Bambusoideae. Monophyly of the true bamboos (i.e., olyroid + woody bamboos) is supported by molecular data in this and other analyses (Clark et al., 1995; Zhang, 1996; Zhang & Clark, 2000). Morphologically, secondary gain of the pseudopetiole (char. 7) and secondary loss of the lamina of the first seedling leaf (char. 41) are synapomorphies. Although only presence or absence of arm cells was scored in this analysis, Zhang and Clark (2000) found that the presence of strongly asymmetrically invaginated arm cells (Fig. 7A) is a potential synapomorphy for this clade. Fusoid cells are characteristic of the Bambusoideae (Fig. 7A), but it is not known whether their presence represents retention of the plesiomorphic condition or reversal after loss of fusoid cells at the base of the BEP Clade. Bambuseae are here supported by the presence of perennating woody culms (char. 1), abaxial ligules (char. 5), and *Panicum*-type starch grains (char. 40). A secondary gain of the inner stamen whorl (chars. 23 and 24) occurred at least once but possibly several times. Olyreae have a synapomorphic base chromosome number of $x = 11$ (char. 47), but the tribe is also characterized by unisexual spikelets.

Ehrhartoideae. This lineage is strongly supported by molecular data, and is characterized by the presence of one female-fertile floret per spikelet, often with one or two proximal female-sterile florets (char. 11). This character is coded as ambiguous in *Oryza* and *Leersia*, but if the vestigial structures at the base of the spikelets in these genera are interpreted as highly reduced glumes, then the presence of proximal female-sterile florets is an unambiguous synapomorphy. Two lodicules (char. 19) are found in this clade; in addition, the inner whorl of stamens (chars. 23 and 24) is regained, styles are not fused (char. 28), and fusoid cells (char. 46) are lost.

Pooideae. Monophyly of the pooid clade is strongly supported by molecular data including cpDNA restriction site data (Soreng et al., 1990; Davis & Soreng, 1993; Nadot et al., 1994; Soreng & Davis, 1998, 2000). Parallel-sided subsidiary cells, lack of microhairs, nonvascularized lodicules (Fig. 6D), and the presence of an epiblast and lack of a scutellar cleft in the embryo (Fig. 6J) are characteristic of a majority of the subfamily but do not constitute unequivocal synapomorphies. In this analysis, the loss of stylar fusion (char. 28) is an unreversed synapomorphy for the Pooideae. Loss of the scutellar tail (char. 36) is widespread in the clade, but polymorphisms prevent its unambiguous optimization. An unreversed transformation to faint or absent vascularization of the lodicules (char. 22) occurs within the Pooideae after the divergence of *Brachyelytrum*. A transformation to the embryonic leaf margins meeting (as opposed to overlapping; Fig. 6L) also occurs after the divergence of *Brachyelytrum* but is reversed in *Phaenosperma* (or *Phaenosperma* + *Anisopogon*). Multicellular microhairs (char. 43) are known only in *Lygeum* + *Nardus*; although this character is scored only for the abaxial leaf surface, it appears that Pooideae, at least above this divergence, are the only group of grasses to lose completely the ability to make multicellular microhairs anywhere on the plant (except possibly the lodicules). Chromosomal evolution in Pooideae is complex (see char. 47), but $x = 12$ is apparently plesiomorphic in the BEP Clade, so numbers such as $x = 10$ and $x = 11$ in the earlier-diverging lineages of Pooideae may well be derived from this condition. The presence of $x = 12$ in *Phaenosperma*, *Ampelodesmos*, and some Stipeae may be a retention; $x = 7$ is clearly a synapomorphy of the core Pooideae (here represented by *Brachypodium*, *Avena*, *Bromus*, and *Triticum*). Two lodicules (char. 19) are found at the base of Pooideae, but a reversal to three occurs in Stipeae (in which another transformation, to two, occurs in *Nassella*); this is undoubtedly an oversimplification of the pattern in the Stipeae in which lodicule number varies considerably (Vickery et al., 1986). Loss of the distally membranous portion of the lodicule (char. 21) is a synapomorphy for Meliceae. The earliest-diverging lineages of the pooid clade have one floret per spikelet (char. 12) (although a rachilla extension is present in *Brachyelytrum*), multiple florets appear in Meliceae, single florets characterize the (*Phaenosperma* + *Anisopogon*) + Stipeae clade, multiple florets are found at the base of the core pooids, and many taxa within the core pooids have one floret per spikelet. Multiple independent origins of multiple florets per spikelet can be hypoth-

esized, but subsequent reduction to one floret per spikelet has clearly occurred in several groups. Patterns of divergence within this clade are complex and still are being evaluated, so some inferences regarding character evolution are likely to change.

The PACCAD Clade. Over half the species of the grass family are included in this clade. Even as early as the 1930s (Avdulov, 1931; Prat, 1932, 1936; Roshevits, 1937), taxa of this clade have been grouped together. Hilu and Wright (1982) were the first to retrieve this clade in a formal analysis, and subsequently support for the monophyly of the clade is found in all molecular analyses to date with sufficient sampling (Hilu & Esen, 1988; Hilu & Johnson, 1991; Davis & Soreng, 1993; Nadot et al., 1994; Barker et al., 1995; Clark et al., 1995; Duvall & Morton, 1996; Liang & Hilu, 1996; Mathews & Sharrock, 1996; Soreng & Davis, 1998; Hsiao et al., 1999; Mathews et al., 2000) except for Cummings et al. (1994), in which an oryzoid clade nested within the PACC clade. Davis and Soreng (1993) named this the PACC clade based on the four subfamilies that were then recognized as comprising the clade, but we here modify the name to reflect the recognition of two additional subfamilies, the Aristidoideae and the Danthonioideae.

The PACCAD Clade is robustly supported based on molecular data and additionally is supported by the presence of an elongated mesocotyl internode (char. 37) and the loss of the epiblast (char. 35; Fig. 6I). The latter character reverses in the clade, so that secondary gain of the epiblast is an apparent synapomorphy for Centhothecoideae. Two characters (chars. 21 and 50) are possible synapomorphies for the PACCAD Clade, but because of a lack of data or lack of a structure in *Micraira*, placement of these transitions is ambiguous. The lack of lodicules in *Micraira* prevents unambiguous placement of the loss of the distally membranous portion of the lodicule (char. 21), and *Micraira* remains unsampled for the presence or absence of the 3 bp deletion in phytochrome B (char. 50). Solid culm internodes (char. 2) are shown here as synapomorphic, although hollow ones reappear in other members of the clade. Non-linear hila (char. 33; Fig. 6M) are widespread in the PACCAD Clade, but the point of origin is ambiguous. The *Panicum*-type starch grain syndrome (char. 40) may be a synapomorphy for the PACCAD Clade, with a reversal to the *Festuca*-type in the clade containing *Eriachne*, Aristidoideae, Danthonioideae, Arundinoideae, and Chloridoideae (the Ligule of Hairs Clade, as defined below), but other optimizations are possible. Two lodicules (char. 19) are established for the BEP + PACCAD Clade, but no transformations to any other number occur in the PACCAD Clade.

The positions of *Micraira* and *Eriachne* in the phylogeny are not well resolved, presumably due to a lack of sequence data for both genera (see Results and also Unresolved Questions). The two species of *Eriachne* are from quite distinct parts of the genus, based on the informal classification of Lazarides (1995). This undoubtedly affects the interpretation of character state transformations within the entire clade.

Early in the evolution of the PACCAD Clade, some lineages developed the capacity for C_4 photosynthesis, apparently as an adaptation to high light/high temperature conditions and perhaps also to falling levels of atmospheric CO_2 (Sage & Monson, 1999). Most members of the Panicoideae, all but two Chloridoideae, the Aristidoideae (except for *Sartidia*), and the Eriachneae are C_4. The poor resolution of the phylogeny at the base of the PACCAD Clade makes it impossible to determine precisely how many origins of C_4 photosynthesis there were, but certainly there were at least two, and possibly more. The data are also consistent with a polymorphism at the base of the PACCAD Clade.

The Panicoideae + Centothecoideae Clade. This clade was recovered in virtually all subanalyses, and had reasonable support (bts 85, brs 8) in the combined analysis. The presence of non-linear hila (char. 33; Fig. 6M) is a potential synapomorphy for this clade. Although support for the monophyly of Panicoideae (excluding *Gynerium* and *Danthoniopsis*) was strong (see Results), relationships of the centothecoid taxa, *Gynerium*, and *Danthoniopsis* to the Panicoideae and to each other remain unresolved, but the placement of *Gynerium* as sister to traditional Panicoideae is a novel result.

Panicoideae. The presence of proximal female-sterile florets (char. 11) and the transformation to the classical NADP-ME C_4 subtype (char. 48) are unambiguous synapomorphies for *Danthoniopsis* + Panicoideae. Some reversions to the C_3 pathway occur within the Paniceae among unsampled taxa, and at least one secondary transformation to the NAD-ME C_4 subtype occurs in *Panicum*. This clade is also supported by the presence of one female-fertile floret (char. 12) as a reversal and the gain of a germination flap (char. 17), but the placement of this latter transformation is ambiguous. The loss of disarticulation above the glumes (char. 16) is a synapomorphy for Panicoideae excluding *Danthoniopsis*. The presence of paired spikelets (char. 9) is a synapomorphy of Andropogoneae in this

analysis, but paired spikelets do occur within Paniceae (e.g., *Brachiaria, Digitaria, Paspalum*).

Centothecoideae. Monophyly of this subfamily as currently constituted is not strongly supported in this analysis. The secondary gain of an epiblast (char. 35) is a possible synapomorphy (but is unknown for *Thysanolaena*), as is fusion of the styles (char. 28).

The Ligule of Hairs Clade (Eriachne + {{*Aristidoideae* + *Danthonioideae*} + {*Arundinoideae* + *Chloridoideae*}}). The adaxial ligule as a fringe of hairs (char. 4), awned lemmas (char. 13), and compound starch grains (char. 40) are synapomorphies of this clade, but characters 4 and 13 reverse multiple times, and character 40 once, within this clade, as well as elsewhere on the tree (Fig. 3). The recovery of this clade is a novel finding, but further investigation is warranted, given the lack of sequence data for *Eriachne*. The transformation to embryonic leaf margins meeting (char. 38; Fig. 6L) is an unreversed synapomorphy of the four subfamilies above *Eriachne*.

The Aristidoideae + Danthonioideae Clade. Although each of these subfamilies is well supported as monophyletic, their sister relationship is another novel result, and one that is only moderately supported. Nonetheless, the presence of a basic pattern of three awns (char. 14; Fig. 5C and F) is an unreversed synapomorphy for this clade. Reappearance of the distal membranous portion of the lodicules (char. 21) also may be a synapomorphy, although this reverses within the Danthonioideae.

Aristidoideae. Gain of a germination flap (char. 17) and transformation to a base chromosome number of $x = 11$ (char. 47) are unambiguous synapomorphies for the clade.

Danthonioideae. The presence of haustorial synergids (char. 32) is interpreted as an unreversed synapomorphy, but wider sampling within the clade is needed.

Arundinoideae. No unambiguous morphological support for the monophyly of this subfamily was found, although a reversal to hollow culms (char. 2) occurs in this clade.

Chloridoideae. Chloridoideae, including *Centropodia* and *Merxmuellera rangei*, are supported based on molecular data, although no clearcut morphological synapormorphies have been identified. Monophyly of *Centropodia* + *M. rangei* is well supported as is that of the traditional Chloridoideae (i.e., Chloridoideae s. str.), but support for the sister relationship of these two clades is relatively modest. The gain of the NAD-ME C_4 subtype (char. 48) is a possible synapomorphy for the entire clade, however, and if so it would then revert to C_3 in *M. rangei*. The gain of chloridoid-type microhairs (char. 44) is a synapomorphy for the traditional Chloridoideae, although the character does occur elsewhere in the PACCAD Clade, and many genera of chloridoids also include species with panicoid-type microhairs (Jacobs, 1987; Van den Borre, 1994; Van den Borre & Watson, 1994). The pericarp is often free or loose, but this feature is not uniform and is also found in non-chloridoid grasses.

UNRESOLVED QUESTIONS

Monophyly of Anomochlooideae. *Anomochloa* and *Streptochaeta* are each distinctive genera but appear to have little in common. We have not yet found a uniquely derived morphological character that unites them as members of a single clade. Although the analyses presented here indicate that the two form a monophyletic group, analyses of single data sets sometimes show them to be paraphyletic or unresolved (Mathews et al., 2000; Hilu et al., 1999; Zhang, 2000). Because both genera occupy long branches in gene trees, they may form a clade only because of long-branch attraction (Felsenstein, 1978). Molecular studies of other species of *Streptochaeta* would help break up the long branch to *S. angustifolia* and might affect the monophyly of the clade. Resolution of *Anomochloa* and *Streptochaeta* as two separate basal lineages obviously would affect interpretations of character evolution within the family.

Position of Streptogyna. As noted in Results, the position of *Streptogyna* is ambiguous, apparently caused by lack of data. There are two species in the genus, one in the New World tropics and the other in Africa. Neither has been collected frequently, and we do not know of any plants in cultivation. Morphologically, the genus would fit comfortably within the Bambusoideae, but molecular data suggest that it is an early-diverging member of the BEP Clade or the Ehrhartoideae. The characters it shares with Bambusoideae are thus presumably ancestral, not indicative of relationship. Accurate placement of *Streptogyna* is necessary for interpretation of character evolution in the early-diverging members of Bambusoideae, Ehrhartoideae, and Pooideae.

Early-diverging Pooideae. The combined analysis confirms the position of *Brachyelytrum* as sister

to the rest of the Pooideae, and *Lygeum* + *Nardus* as the next-diverging lineage; both these results are well supported. The next diverging lineages include *Phaenosperma*, *Anisopogon*, Stipeae, *Ampelodesmos*, Meliceae, and Diarrheneae, but the order of divergence is not resolved by any data collected to date. In the case of *Phaenosperma*, *Anisopogon*, *Diarrhena*, and *Ampelodesmos*, the problem may be ascribed to insufficient sequence data in this analysis. For our sample of Stipeae and Meliceae, however, appreciable sequence data are available, yet the relative positions of the two lineages remain unclear. If the phylogenetic problem is indeed soluble with molecular data, the sample of genera and species in each tribe may have to be increased substantially. A combined analysis of cpDNA restriction sites and morphology (Soreng & Davis, 2000) represents the broadest taxon sample for Pooideae among studies to date. The order of divergence of these lineages affects interpretation of the evolution of such characters as parallel-sided subsidiary cells, loss of microhairs, and trends in reduction of chromosome number (Kellogg, 1998). The latter may correlate with a marked increase in genome size (Bennetzen & Kellogg, 1997) and may suggest possible mechanisms of genome evolution.

The PACCAD Clade. Relationships among the major lineages in the PACCAD Clade are not resolved by this or any other phylogenetic analysis to date. In the combined analysis, the branches at the base of the clade are short, marked by relatively few mutations each (11, 41, and 16 steps; Fig. 1). This suggests that the PACCAD radiation may have occurred relatively rapidly. If this is true, then relationships may remain difficult to resolve with certainty. The clade also contains a number of taxa of uncertain placement, many of which have received little or no attention in phylogenetic studies. The tribe Eriachneae, which includes the Australian genera *Eriachne* and *Pheidochloa*, is represented here only by an *rbc*L sequence of *Eriachne triodioides* and an ITS sequence of *E. triseta*. The genus *Micraira*, the only member of the Australian tribe Micraireae, is represented only by an *ndh*F sequence of *M. lazaridis* and by an ITS sequence of *M. subulifolia*. Such genera as *Cyperochloa*, *Steyermarkochloa*, and the *Crinipes* group were not included in this combined analysis. An *rbc*L sequence of *Cyperochloa* places it with the centothecoids, whereas a sequence of the crinipoid genus *Styppeiochloa* places it sister to Arundineae s. str. (Barker, 1997; Linder et al., 1997).

The subfamilies recognized within the PACCAD Clade are, except for Centothecoideae, strongly supported as monophyletic by our data. The list of genera included in and excluded from each subfamily, however, is based on a rather limited sample of species and genera, combined with inferences from classical morphological studies. In particular, the exact circumscriptions of Danthonioideae, Arundinoideae, and Centothecoideae are not precisely determined by this study. A comprehensive effort by multiple systematists is needed to improve understanding of the many poorly known species and genera.

Centothecoideae. Of the groups recognized here as subfamilies, Centothecoideae are the only one not strongly supported as monophyletic by the combined analysis. We have retained the subfamilial name and expanded the circumscription to include *Thysanolaena*, formerly a member of the Arundinoideae. As with the remainder of the PACCAD Clade, a clear picture of the limits of the centothecoid clade depends on much more data, particularly on the remaining centothecoid genera, but a study is under way (J. G. Sánchez-Ken, pers. comm.).

Circumscription of tribes. This paper does not address tribal circumscription. This will require far more extensive sampling, particularly in Pooideae, Panicoideae, Chloridoideae, and Bambusoideae, which constitute the four largest subfamilies. Choice of outgroups for such studies is now clear, however.

Biogeography. Present-day distributions do not indicate much about where the grasses originated. Restionaceae are clearly a Gondwanan group, with representatives in Africa and Australia. Joinvilleaceae, however, are insular, occurring on Borneo, New Caledonia, and Pacific Islands. The basal lineages of the grasses are found in the tropical regions of South America, Africa, and Asia; the Anomochlooideae are restricted to South and Central America (Judziewicz & Soderstrom, 1989), the Pharoideae are pantropical (Soderstrom et al., 1987), and the Puelioideae are restricted to tropical Africa (Soderstrom & Ellis, 1987; Clark et al., 2000). Due to the absence of an early fossil record, it is not clear how this distribution was established, whether by long-distance dispersal across the Atlantic and Indian Oceans, or whether across a continuous Gondwanan equatorial forest. Either way, the continent of origination cannot be determined with current data.

Timing and causes of diversification. The earliest unequivocal grass fossils are pollen grains from the Paleocene of South America and Africa,

deposited approximately 60 to 55 million years (my) ago (Jacobs et al., 1999), although some grains of *Monoporites* from the upper Maastrichtian (Cretaceous) may also represent remains of grasses (Linder, 1987). The earliest known grass macrofossil appears in an early Eocene formation (ca. 55 mya) in North America (Crepet & Feldman, 1991). Based on the fossil record therefore, the family is at least 55 my and possibly as much as 70 my old. Establishment of all major lineages had occurred by the mid-Miocene (Jacobs et al., 1999), which is about the time that grass-dominated ecosystems appeared.

Attempts to date particular nodes on the cladogram using molecular clocks are confounded by non-clocklike behavior of several of the genes (Gaut et al., 1996, 1997; Mathews et al., 2000; Kellogg & Russo, unpublished obs.). Using sequences of GBSSI, which has been shown to exhibit clocklike mutation, Gaut and Doebley (1997) placed the divergence of maize and *Pennisetum* at 25 mya, whereas Kellogg and Russo (unpublished) place the divergence of *Danthoniopsis dinteri* from the rest of the panicoids at ca. 16 mya. The two dates conflict with each other, but do suggest that the PACCAD Clade originated in the early Miocene or late Oligocene.

All C_4 lineages are included in the PACCAD Clade, so paleontological evidence for C_4 photosynthesis can establish a minimum age for the common ancestor of the clade. The earliest known C_4 grass macrofossil is dated at 12.5 mya (Nambudiri et al., 1978), and the earliest isotopic evidence for C_4 is ca. 15 mya (Kingston et al., 1994; Latorre et al., 1997). This suggests that the origin of the PACCAD Clade occurred no later than 15 mya and possibly as early as 25 mya.

Both fossil data and molecular clock estimates seem at odds with the apparent Gondwanan distribution of many grass taxa (see for example Simon & Jacobs, 1990). The Gondwanan distribution of such derived groups as the subfamily Danthonioideae might suggest that the PACCAD Clade originated sometime before the breakup of Gondwana, which would then place the origin of the family long before the earliest known fossils were deposited. This cannot be ruled out, of course, because it is an assumption based on negative evidence. If, however, we assume that groups within the PACCAD Clade originated before the breakup of Gondwana (a process hard to date precisely but perhaps 100–70 mya), then we would have to assume that the family originated more than 200–140 mya, before the time of the first appearance of angiosperms in the fossil record. It seems more likely, and more consistent with available data, that the grasses achieved their Gondwanan distribution by dispersal (Soreng & Davis, 1998), as has been suggested for other taxa with an apparent Gondwanan distribution (e.g., *Adansonia*, Baum et al., 1998; Atherospermataceae, Renner et al., 2000).

The combination of fossil data and molecular clock evidence suggests that the major diversification of the grasses occurred between 15 and 25 mya, long after the origin of the family at 55 to 70 mya. This is consistent with the observed branch lengths on the phylogeny in Figure 1. There may have been many more representatives of the Anomochlooideae, Pharoideae, and Puelioideae (or even additional lineages) that are now extinct, but grasses are generally rare in the fossil record until the Miocene (Jacobs et al., 1999). The simplest explanation is that the family diversified long after its origin. The novel characters that arose after the divergence of *Joinvillea*—the caryopsis, differentiated embryo, reduction in perianth—therefore did not lead immediately or directly to the current dominance of the family. Other characteristics acquired later in the evolution of the family may have been more important in its diversification and ecological success. Possibilities include such characters as formation of intercalary meristems or the acquisition of mechanisms for drought tolerance. We do not know the phylogenetic distribution of intercalary meristems, however, and it is possible that intercalary meristems of the leaves evolved after such meristems in the stems. This character needs to be investigated further. Acquisition of drought and heat tolerance would also be worth investigating, but would require a precise definition of what is meant by each term. The cellular components of such physiological responses are being identified and could perhaps be studied across a range of taxa.

CONCLUSIONS

We present here a resolved and strongly supported phylogeny of the grass family. It can be used to understand the diversification of morphology, genes, and genomes, to interpret comparative studies of cereal crops and forage grasses, and to develop hypotheses of adaptation to past and future environments. Some phylogenetic questions remain unresolved, and these affect inferences about such important characters as C_4 photosynthesis. Nonetheless, this phylogeny is one of the most comprehensive and robust available for any family of plants, making the grasses an excellent clade for studies of evolutionary pattern and process.

Taxonomic Treatment

Twelve subfamilies are recognized formally in this classification system (Table 1). A description is provided for each subfamily, and where appropriate, synonymy is indicated. To permit easy comparison with previous work, we have listed for each subfamily which of the tribes recognized by Clayton and Renvoize (1986) are to be included. In some cases (e.g., Pharoideae or Danthonioideae), the new circumscription of subfamilies makes tribal recognition largely unnecessary. For example, the subfamily Pharoideae includes three genera in a single tribe; the tribe is effectively redundant and serves no useful function in the subfamilial classification. Nonetheless we list the names for comparison.

Our sample of taxa was explicitly designed to explore relationships among major clades that can be recognized at the subfamilial level, but it is not dense enough to evaluate tribal limits. We have in many cases combined molecular data from several species to represent a genus (as is also commonly done for morphological analyses), and in a few cases have combined data from several genera that represent a putatively monophyletic group. Such combinations assume, rather than test, monophyly. We therefore refrain from formal discussion of tribal limits, which cannot be addressed by our data; these limits will have to be re-evaluated by future studies. Three tribes and two genera are placed as Incertae Sedis at the end of the classification, although the genera may be provisionally placed as noted below.

This classification reflects our attempt to use the phylogeny as the basis for recognizing subfamilies while remaining nomenclaturally conservative. Except for Centothecoideae, all subfamilies recognized are well supported as monophyletic in our analyses. While we could create an unranked classification for the grasses using our phylogeny, we feel that the practical interests of the potential users of this classification currently are best served by retaining the Linnaean hierarchy. Nonetheless we have applied informal names to several of the well-supported clades (see above).

The most significant changes in our proposed subfamily classification are the breakup of the traditional Bambusoideae and Arundinoideae and the expansion of Pooideae. The diversity encompassed by the traditional Bambusoideae (or Bambusoideae s.l.) is now recognized as Anomochlooideae, Pharoideae, Puelioideae, Bambusoideae s. str., and Ehrhartoideae. Elements of the traditional Arundinoideae are now recognized as Aristidoideae, Danthonioideae, and Arundinoideae s. str., with Thysanolaeneae placed in the Centothecoideae and *Gynerium* as Incertae Sedis. *Centropodia* and *Merxmuellera rangei* are placed in Chloridoideae. Pooideae have grown by inclusion of Brachyelytreae, Lygeeae, Nardeae, Phaenospermatideae, Diarrheneae, Stipeae, and Ampelodesmeae, all formerly classified within either Bambusoideae or Arundinoideae by some authors; note, however, that Clayton and Renvoize (1986) placed Lygeeae, Nardeae, and Stipeae in Pooideae in agreement with the classification proposed here. A detailed comparison of the GPWG classification with the major grass classification systems of the 20th century is presented in Table 1.

Primary sources for suprageneric names were the STAR Database (http://matrix.nal.usda.gov:8080/star/supragenericname.html), the Catalog of New World Grasses (http://mobot.mobot.org/W3T/Search/nwgc.html), and Clayton and Renvoize (1986). Diagnoses of the subfamilies were extracted from various sources including Clayton and Renvoize (1986) and Watson and Dallwitz (1992). Tribes in Chloridoideae and Panicoideae (except for the exclusion of Eriachneae) follow the treatment of Clayton and Renvoize (1986); tribes listed for the other subfamilies generally are treated according to more recent studies and/or consultation with specialists in those groups.

Poaceae (R. Br.) Barnh., Bull. Torrey Bot. Club 22: 7. 1895. (Nom. alt. Gramineae Juss., Gen. Pl.: 28. 1789.)

A monophyletic family, recognizable by the following synapomorphic morphological characters: Inflorescence highly bracteate. Perianth reduced or lacking. Pollen lacking scrobiculi, but with intraexinous channels. Seed coat fused to inner ovary wall at maturity, forming a caryopsis. Embryo highly differentiated with obvious leaves, shoot and root meristems, and lateral in position.

1. **Anomochlooideae** Pilg. ex Potztal, in Willdenowia 1: 772. 1957. TYPE: *Anomochloa* Brongn. Figure 4C and D.

Syn.: Streptochaetoideae (Nakai) Butzin, Neue Unters. Blüte Gram.: 148. 1965.

Plants perennial, rhizomatous, herbaceous, of shaded tropical forest understories. Culms hollow or solid. Leaves with phyllotaxis either distichous or spiral; abaxial ligule absent; adaxial ligule a short fringe of cilia or absent, not membranous; blades usually relatively broad, venation parallel, with pseudopetioles short to very long, these with dark, turgid swellings (pulvini) at both ends (*An-*

omochloa) or only at the summit (*Streptochaeta*); sheaths non-auriculate. Inflorescences spicate, with complicated branching patterns, bracts outside of the spikelet equivalents present, large and with a blade or small and bladeless, or absent. Ultimate structures of the inflorescence (spikelet equivalents) of uncertain homology with typical grass spikelets but one-flowered and bisexual; bracts within the spikelet equivalents with phyllotaxis distichous or spiral, lacking uncinate macrohairs, sometimes awned but if so, the awns single; lodicules absent, or, in *Anomochloa*, their position occupied by a ring of short brownish cilia borne on a low membranous ring; stamens 4 or 6; ovary glabrous, apical appendage absent, haustorial synergids presumed absent, style 1, stigma(s) 1 or 3. Caryopsis with the hilum linear, shallow and inconspicuous; endosperm hard, containing compound starch grains; embryo large, epiblast present or not, scutellar cleft present but shallow, mesocotyl internode absent, embryonic leaf margins overlapping or not. Basic chromosome numbers: $x = 11$ or 18 (note: Clark & Judziewicz, 1996, erroneously cited these as 12 or 18).

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer absent, with fusoid cells very large and well developed, arm cells only weakly developed; Kranz anatomy absent; midrib complex; adaxial bulliform cells present.

Foliar micromorphology. Stomata with low dome-shaped and triangular subsidiary cells; bicellular microhairs very large (0.075–0.15 mm), the pointed apical cell usually one and a half times as long as the basally constricted basal cell; papillae absent.

Photosynthetic pathway. Presumed C_3.

INCLUDED TRIBES:

Anomochloeae C. E. Hubb., in Hutchinson, Fam. Fl. Pl. 2: 219. 1934. TYPE: *Anomochloa* Brong.

Streptochaeteae C. E. Hubb., in Hutchinson, Fam. Fl. Pl. 2: 205. 1934. TYPE: *Streptochaeta* Schrad. ex Nees.

Notes. There is no unique morphological synapomorphy for this subfamily, but both tribes lack lodicules and they apparently also lack grass-type spikelets. As noted above (Unresolved Questions), this lineage may not be monophyletic, in which case two subfamilies would need to be recognized. The subfamily includes 4 species.

II. Pharoideae (Stapf) L. G. Clark & Judz., Taxon 45: 643. 1996. TYPE: *Pharus* P. Browne. Figure 4B.

Syn.: Leptaspidoideae (Tzvelev) C. O. Morales, Sendtnera 5: 244. 1998. Nom. superfl.

Plants perennial, rhizomatous, monoecious, herbaceous, of shaded tropical to warm temperate forest understories. Culms hollow or solid. Leaves distichous; abaxial ligule absent; adaxial ligule a fringed membrane; blades resupinate, relatively broad, with pseudopetioles prominent and twisted, with lateral nerves diverging obliquely from midnerve and running straight to margins; sheaths non-auriculate. Inflorescences paniculate, the main axis and branches disarticulating or not, covered with uncinate macrohairs, bracts outside of the spikelets absent. Spikelets unisexual, one-flowered, mostly in male-female pairs on short branchlets, or some female spikelets solitary. Female spikelets large, short-stalked; glumes 2, shorter than the floret; lemma tubular or inflated, covered wholly or in part by uncinate macrohairs, awnless; palea well developed; lodicules absent; ovary glabrous, apical appendage absent, haustorial synergids presumed absent, style 1, stigmas 3. Caryopsis with the hilum linear, extending the full length; endosperm hard, without lipid; embryo small, epiblast present, scutellar cleft present but shallow, mesocotyl internode absent, embryonic leaf margins overlapping. Male spikelets small, short- to long-stalked, membranous; glumes 2, shorter than the floret; lodicules 3 or 0, if present then minute, elliptic, glabrous, and nerveless; stamens 6. Basic chromosome number: $x = 12$.

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells large and well developed, arm cells weakly to moderately well developed; Kranz anatomy absent; midrib complex; inflated adaxial interstomatal cells present, bulliform cells poorly developed or absent.

Foliar micromorphology. Stomata with parallel-sided to dome-shaped subsidiary cells; bicellular microhairs and papillae absent.

Photosynthetic pathway. Presumed C_3.

INCLUDED TRIBE (NOW IDENTICAL TO SUBFAMILY AND THUS REDUNDANT):

Phareae Stapf, in Thiselton-Dyer, Fl. Cap. 7: 319. 1898. TYPE: *Pharus* P. Browne.

Notes. In his original description of the tribe, Stapf specifically included *Olyra* (based on its uni-

sexual spikelets), but did not explicitly list *Pharus* or *Leptaspis*, although his choice of the name Phareae implicitly recognized the membership of *Pharus* in the tribe and automatically placed *Pharus* as its type, according to Article 10.6 of the *Code* (Greuter et al., 2000). As long as *Olyra* was retained in the same tribe as *Pharus*, Phareae was a superfluous name for the Olyreae. When *Pharus* and *Leptaspis* are segregated into their own tribe, and *Olyra* is excluded, then Phareae becomes the valid, correct name for the tribe. Clark and Judziewicz (1996) based the name of the subfamily on this tribal name. Tzvelev (1989) argued that the name Phareae was illegitimate because the type of the previously described tribe Olyreae was included in it, and provided the name Leptaspideae for this tribe. Morales (1998) agreed with Tzvelev and rejected the name Pharoideae for this subfamily, according to Article 52.1 of the *Code* (Greuter et al., 2000), replacing it with Leptaspidoideae. Under Article 52.3, however, "A name that was nomenclaturally superfluous when published is not illegitimate . . . if it is based on the stem of a legitimate generic name." We therefore accept the name Pharoideae for this subfamily, as *Pharus* is a legitimate generic name. The subfamily includes 12 species.

III. Puelioideae L. G. Clark, M. Kobay., S. Mathews, Spangler & E. A. Kellogg, Syst. Bot. 25: 181–187. 2000. TYPE: *Puelia* Franch. Figure 4A.

Plants perennial, rhizomatous, herbaceous, of shaded rainforest understories. Culms hollow. Leaves distichous; abaxial ligule absent (*Guaduella*) or present (*Puelia*); adaxial ligule a fringed membrane; blades relatively broad, pseudopetiolate, venation parallel; sheaths non-auriculate. Inflorescences racemose or paniculate, bracts outside of the spikelets sometimes present. Spikelets with two glumes and several florets, the 1 to 3 proximal florets male, the next several florets female-fertile, with distal incomplete florets (*Guaduella*), or the proximal 3 to 6 florets male or neuter with the single distal floret female (*Puelia*), disarticulating above the glumes and between the florets (*Guaduella*) or not (*Puelia*); lemmas lacking uncinate macrohairs, awnless; palea well developed, sometimes tubular; lodicules 3, membranous, ciliate; stamens 6; ovary glabrous or hairy, an apical appendage present or not, haustorial synergids presumed absent, styles 2 or 3, the bases close, stigmas 2 or 3. Caryopsis with a long-linear hilum; embryo small. Basic chromosome number: $x = 12$.

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells well developed, arm cells only weakly developed; Kranz anatomy absent; midrib complex or less commonly simple; adaxial bulliform cells present.

Foliar micromorphology. Stomata with dome-shaped to triangular subsidiary cells; microhairs absent (*Puelia*) or multicellular, uniseriate microhairs present (*Guaduella*); papillae present or more commonly absent.

Photosynthetic pathway. Presumed C_3.

INCLUDED TRIBES:

Guaduelleae Soderstr. & R. P. Ellis, in Soderstrom et al. (editors), Grass Syst. Evol.: 238. 1987. TYPE: *Guaduella* Franch.

Puelieae Soderstr. & R. P. Ellis, in Soderstrom et al. (editors), Grass Syst. Evol.: 238. 1987. TYPE: *Puelia* Franch.

Notes. This subfamily, which comprises approximately 14 species, is poorly known, and morphological, anatomical, cytological, and ecological studies are needed.

IV. Bambusoideae Luerss., Grundz. Bot., ed. 5: 451. 1893. TYPE: *Bambusa* Schreb. Figures 4F, 6C, G, O, P, 7A.

Syn.: Olyroideae Pilger, Nat. Pfl.-Fam. ed. 2, 14d: 168. 1956.
Parianoideae (Nakai) Butzin, Neue Unters. Blüte Gram.: 148. 1965.

Plants perennial (rarely annual), rhizomatous, herbaceous or woody, of temperate and tropical forests, tropical high montane grasslands, riverbanks, and sometimes savannas. Culms hollow or solid. Leaves distichous; abaxial ligule absent (Olyreae) or present (Bambuseae); adaxial ligule membranous or chartaceous, fringed or unfringed; blades usually relatively broad, pseudopetiolate, venation parallel; sheaths often auriculate. Inflorescences spicate, racemose or paniculate, completing development of all spikelets in one period of growth and subtending bracts and prophylls usually absent, or pseudospikelets with basal bud-bearing bracts producing two or more orders of spikelets with different phases of maturity and subtending bracts and prophylls usually present. Spikelets (or spikelets proper of the pseudospikelets) bisexual (Bambuseae) or unisexual (Olyreae), consisting of 0, 1, 2 or several glumes, 1 to many florets; lemma lacking uncinate macrohairs, if awned, the awns single; palea well developed; lodicules usually 3 (rarely 0 to 6 or many), membranous, vascularized, often ciliate; stamens usually 2, 3, or 6 (10 to 40 in *Pariana*, 6

to 120 in *Ochlandra*); ovary glabrous or hairy, sometimes with an apical appendage, haustorial synergids absent, styles 2 or 3, sometimes very short but close, stigmas 2 or 3. Caryopsis with hilum linear (or rarely punctate), extending its full length (or rarely less than full length); endosperm hard, without lipid, containing compound starch grains; embryo small, epiblast present, scutellar cleft present, mesocotyl internode absent, embryonic leaf margins overlapping. Basic chromosome numbers: $x = 7, 9, 10, 11$, and 12.

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells large and well developed, arm cells usually well developed and strongly invaginated; Kranz anatomy absent; midrib complex or simple; adaxial bulliform cells present.

Foliar micromorphology. Stomata with dome-shaped, triangular, or parallel-sided subsidiary cells; bicellular microhairs present, panicoid-type; papillae common and abundant.

Photosynthetic pathway. C_3.

INCLUDED TRIBES:

Bambuseae Dumort., Anal. Fam. Pl.: 63. 1829. TYPE: *Bambusa* Schreb.

Olyreae Kunth ex Spenn., Fl. Friburg. 1: 172. 1825. TYPE: *Olyra* L. (Including Buergersiochloeae Blake, Blumea, Suppl. 3: 62. 1946; Parianeae C. E. Hubbard, in Hutch., Fam. Fl. Pl. 2: 219. 1934.)

Notes. The current circumscription of this subfamily is much narrower than the traditional view. In their recent analysis, Zhang and Clark (2000) recovered two robustly supported clades, the olyroid bamboos and the woody bamboos, which they recognized as tribes Olyreae and Bambuseae, respectively. Following Zhang and Clark (2000), Buergersiochloeae and Parianeae are included in Olyreae. This subfamily includes approximately 1200 species.

V. Ehrhartoideae Link, Hort. Berol. 1: 233. 1827. TYPE: *Ehrharta* Thunb. Figure 4G.

Syn.: Oryzoideae Kunth ex Beilschm., Flora 16(2): 52, 109. 1833.

Plants annual or perennial (rhizomatous or stoloniferous), herbaceous to suffrutescent, of forests, open hillsides, or aquatic habitats. Culms hollow or solid. Leaves distichous; abaxial ligule absent; adaxial ligule a fringed or unfringed membrane, or a fringe of hairs; blades rarely basally cordate or sagittate (Phyllorachideae), somewhat broad to usually narrow, sometimes pseudopetiolate, venation parallel; sheaths sometimes bearing auricles. Inflorescences paniculate or racemose, bracts outside of the spikelets rarely present (*Humbertochloa*). Spikelets bisexual or unisexual, with glumes 2 (absent in some Oryzeae), sterile florets 0 to 2, and female-fertile floret 1, disarticulating above the glumes or infrequently primary branches disarticulating as units; lemma lacking uncinate macrohairs, if awned, the awn single; palea well developed; lodicules 2, membranous or rarely fleshy, heavily vascularized; stamens usually 3 or 6 (sometimes 1, 2, or 4); ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, free, fused basally or for their full length (*Zizaniopsis*), close, stigmas 2. Caryopsis with the hilum long-linear; endosperm hard, without lipid, containing compound starch grains (rarely simple); embryo small, epiblast usually present (absent in *Ehrharta*), scutellar cleft usually present (absent in *Leersia* and *Potamophila*), mesocotyl internode absent (present but short in *Microlaena*), embryonic leaf usually with overlapping margins (meeting in *Potamophila*). Basic chromosome numbers: $x = 12$ (10 in *Microlaena*; 15 in *Zizania*).

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer usually absent, fusoid cells absent or sometimes present (*Zizania* and *Zizaniopsis*), arm cells absent or present; Kranz anatomy absent; midrib simple or complex; adaxial bulliform cells present.

Foliar micromorphology. Stomata with dome-shaped or triangular subsidiary cells; bicellular microhairs present, panicoid-type; papillae often present in Oryzeae, otherwise absent.

Photosynthetic pathway. C_3.

INCLUDED TRIBES:

Ehrharteae Nevski, Trudy Bot. Inst. Akad. Nauk SSSR 4: 227. 1937. TYPE: *Ehrharta* Thunb.

Oryzeae Dumort., Observ. Gramin. Belg.: 83. 1824. TYPE: *Oryza* L.

Phyllorachideae C. E. Hubb., in Hook. Ic. Pl. 34: t. 3386, p. 5. 1939. TYPE: *Phyllorachis* Trimen.

Notes. Although we did not sample Phyllorachideae, we place it here based on morphological similarity. Nonetheless, any future studies of this clade should include this tribe to test its relationship to Ehrharteae and Oryzeae. Under the present circumscription, this subfamily includes approximately 120 species.

VI. Pooideae Benth., Fl. Hongk. 407. 1861. TYPE: *Poa* L. Figures 4E, H, 6D, J, L, 7B.

Syn.: Avenoideae Link, Hort. Berol. 1: 108. 1827.
Festucoideae Link, Hort. Berol. 1: 137. 1827.
Glycerioideae Link, Hort. Berol. 1: 160. 1827.
Echinarioideae Link, Hort. Berol. 1: 197. 1827.
Cynosuroideae Link, Hort. Berol. 1: 198. 1827.
Anthoxanthoideae Link, Hort. Berol. 1: 232, 271. 1827.
Agrostidoideae Kunth ex Beilschm., Flora (Beib.) 16(2): 52, 104. 1833.
Stipoideae Burmeist., Handb. Naturgesch. 199. 1837.
Hordeoideae Burmeist., Handb. Naturgesch. 202. 1837.
Phalaroideae Burmeist., Handb. Naturgesch. 208. 1837.
Secaloideae Rouy, Fl. France 14: 2, 298. 1913.

Plants annual or perennial (rhizomatous, stoloniferous, or neither), herbaceous, of cool temperate and boreal regions, extending across the tropics in the high mountains. Culms hollow (rarely solid). Leaves distichous; abaxial ligule absent; adaxial ligule scarious or membranous, the margin not or infrequently short ciliate fringed (rarely long ciliate, *Anisopogon*); blades somewhat broad to usually narrow, rarely pseudopetiolate (*Phaenosperma*), venation parallel; sheaths sometimes auriculate. Inflorescences spicate, racemose, or paniculate, bracts outside of the spikelets absent or rarely present (e.g., *Sesleria, Echinaria, Ammochloa*). Spikelets bisexual, infrequently unisexual or mixed, usually with two glumes (rarely without glumes, *Lygeum*, or the first absent, *Hainardia, Lolium, Nardus*, except on terminal spikelets), 1 to many female-fertile florets with apical or infrequently basal reductions, compressed laterally, infrequently not or dorsally compressed, disarticulating above the glumes (infrequently below the glumes, some Poeae, or at the nodes of the inflorescence, various genera); lemma lacking uncinate macrohairs, if awned, the awn single; palea usually present and well developed, but variable and sometimes very reduced or absent; lodicules 2 (rarely 3, *Anisopogon*, Ampelodesmeae, many Stipeae and few Poeae; fused, Meliceae; rarely absent, *Lygeum, Nardus*, and few Poeae), usually lanceolate, broadly membranous apically (fleshy, truncate, Meliceae), often lobed (Triticeae, Poeae), obscurely few-nerved, or infrequently ± distinctly few-nerved, not or conspicuously ciliate on the margins; stamens usually 3 (infrequently 1 or 2); ovary glabrous or pubescent, rarely with an apical appendage (*Bromus, Diarrhena*) or rostellum (e.g., *Brachyelytrum, Rostraria*), haustorial synergids absent, styles usually 2, close, stigmas 2 (rarely 1, *Lygeum, Nardus*, and a few others, or 3, scattered genera). Caryopsis with the hilum linear and up to as long as the fruit, or subbasal and punctiform, linear, ellipsoidal, ovate, or circular and less than ⅓ the length of the fruit; endosperm hard or sometimes soft or liquid (some Poeae), with or without lipids (some Poeae), containing compound starch grains, or simple starch grains (Brachyelytreae, Bromeae, Triticeae, some Stipeae); embryo small, epiblast present (rarely absent), scutellar cleft absent (rarely present, but not deeply incised), mesocotyl internode absent (rarely short, *Brachyelytrum*), embryonic leaf margins meeting (infrequently margins overlapping). Basic chromosome numbers: $x = 7$ (Bromeae, Triticeae, Poeae generally, few Brachypodieae), 2, 4, 5, 6, 8, 9, 10, 11, 12, 13 represented in a few Poeae and the other tribes, generally medium or large.

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells absent, arm cells absent; Kranz anatomy absent; midrib simple; adaxial bulliform cells present.

Foliar micromorphology. Stomata with parallel-sided subsidiary cells; bicellular microhairs absent (rarely present, *Lygeum*, where chloridoid, *Nardus*, where panicoid), unicellular microhairs absent (rarely present, few Stipeae); papillae usually absent, when present rarely more than one per long cell.

Photosynthetic pathway. C_3.

INCLUDED TRIBES:

Ampelodesmeae (Conert) Tutin, Bot. J. Linn. Soc. 76: 369. 1978. TYPE: *Ampelodesmos* Link.
Brachyelytreae Ohwi, Bot. Mag. Tokyo 55: 361. 1941. TYPE: *Brachyelytrum* P. Beauv.
Brachypodieae (Hack.) Hayek, Oesterr. Bot. Z. 74(10): 253. 1925. TYPE: *Brachypodium* P. Beauv.
Bromeae Dumort., Observ. Gramin. Belg.: 83. 1824. TYPE: *Bromus* L.
Brylkinieae Tateoka, Canad. J. Bot. 38: 962. 1960. TYPE: *Brylkinia* F. Schmidt.
Diarrheneae (Ohwi) C. S. Campb., J. Arnold Arbor. 66: 188. 1985. TYPE: *Diarrhena* P. Beauv.
Lygeeae J. Presl, Wsobecny Rostl. 2: 1708, 1753. 1846. TYPE: *Lygeum* Loefl. ex L.
Meliceae Link ex Endl., Fl. Poson.: 116. 1830. [as "Melicaceae"] TYPE: *Melica* L.
Nardeae W. D. J. Koch, Syn. Fl. Germ. Helv.: 830. 1837. TYPE: *Nardus* L.
Phaenospermatideae Renvoize & Clayton, Kew Bull. 40: 478. 1985. TYPE: *Phaenosperma* Munro ex Benth.
Poeae R. Br., Voy. Terra Austral. 2: 582. 1814. TYPE: *Poa* L. (Including Aveneae Dumort.,

Observ. Gramin. Belg.: 82. 1824; Agrostideae Dumort., Observ. Gramin. Belg.: 83. 1824.)

Stipeae Dumort., Observ. Gramin. Belg.: 83. 1824 [as "Stipaceae"]. TYPE: *Stipa* L.

Triticeae Dumort., Observ. Gramin. Belg.: 82, 84, 91. 1824. TYPE: *Triticum* L.

Notes. Relationships among some of the major lineages of the core Pooideae clade remain unresolved, and conflicts between molecular data and morphologically based tribal classifications exist (e.g., Poeae vs. Aveneae; see Soreng & Davis, 2000). This is one of several reasons that we do not offer a formal classification of tribes at this point. Relationships among the earlier diverging lineages of the whole pooid clade are only weakly supported, and also require further investigation. The tribal classification presented here is almost certain to change as additional data accumulate, and thus should be taken only as an indication of the taxa included within the subfamily. The subfamily includes approximately 3300 species.

VII. Aristidoideae Caro, Dominguezia 4: 16. 1982. TYPE: *Aristida* L. Figure 5C.

Plants annual or perennial, caespitose, herbaceous, xerophytic or less commonly mesophytic, of temperate, subtropical and tropical zones, often in open habitats. Culms solid or hollow. Leaves distichous; abaxial ligule absent or present as a line of hairs; adaxial ligule a fringed membrane or a fringe of hairs; blades relatively narrow, without pseudopetioles, venation parallel; sheaths non-auriculate. Inflorescences paniculate, bracts outside of the spikelets absent. Spikelets with bisexual florets, glumes 2, female-fertile floret 1, and no rachilla extension, cylindrical or laterally compressed, disarticulating above the glumes; lemma with three awns, the awns separate from each other, or fused below into a twisted column; palea short, less than half the lemma length; lodicules present or rarely absent, when present 2, free, membranous, glabrous, heavily vascularized; stamens 1 to 3; ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, free, close, stigmas 2. Caryopsis with the hilum short or long-linear; endosperm hard, without lipid, containing compound starch grains; embryo small (*Sartidia*) or large (*Aristida, Stipagrostis*), epiblast absent, scutellar cleft present or absent (*Sartidia*), mesocotyl internode elongated, embryonic leaf margins meeting. Basic chromosome numbers: $x = 11, 12$.

Foliar anatomy. Mesophyll radiate or nonradiate (*Sartidia*), an adaxial palisade layer absent, fusoid cells absent, arm cells absent; Kranz anatomy absent (*Sartidia*) or present (*Stipagrostis, Aristida*), when present with one (*Stipagrostis*) or two (*Aristida*) parenchyma sheaths, although both not equally well developed throughout the genus; midrib simple; adaxial bulliform cells present.

Foliar micromorphology. Stomata dome-shaped or triangular; bicellular microhairs present, panicoid-type; papillae absent.

Photosynthetic pathway. C_3 (*Sartidia*); C_4 (*Aristida*, NADP-ME; *Stipagrostis*, not biochemically typed, but anatomically NAD-ME; Hattersley & Watson, 1992).

INCLUDED TRIBE (NOW IDENTICAL TO THE SUBFAMILY AND THUS REDUNDANT):

Aristideae C. E. Hubbard, in Bor, Grasses Burma, Ceylon, India & Pakistan: 685. 1960. TYPE: *Aristida* L.

Notes. The presence of a basal column of the awn is a potential morphological synapomorphy for this clade. *Sartidia* diverges from *Stipagrostis* and *Aristida* in other respects, and should be sampled in future analyses. The subfamily includes approximately 350 species.

VIII. Arundinoideae Burmeist., Handb. Naturgesch.: 204. 1837. TYPE: *Arundo* L. Figure 5A.

Syn.: Phragmitoideae Parodi ex Caro, Dominguezia 4: 13. 1982.

Plants perennial (rarely annual), rhizomatous, stoloniferous, or caespitose, herbaceous to somewhat woody, of temperate and tropical areas, mesophytic or xerophytic, the reeds found in marshy habitats. Culms hollow or less commonly solid. Leaves distichous; abaxial ligule absent or rarely present as a line of hairs (*Hakonechloa*); adaxial ligule a fringed or unfringed membrane or a fringe of hairs; blades relatively broad to narrow, without pseudopetioles, venation parallel; sheaths usually non-auriculate. Inflorescences usually paniculate, rarely spicate or racemose, bracts outside of the spikelets absent. Spikelets with bisexual florets, glumes 2, a sterile lemma sometimes present, female-fertile florets 1 to several, apical reduction usually present, usually laterally compressed, disarticulating above the glumes; lemma lacking uncinate macrohairs, if awned, awn usually single, sometimes awns three, but then lacking a basal column; palea usually well developed; lodicules 2, free (rarely joined at the base), fleshy, glabrous or

infrequently ciliate, not or scarcely vascularized to heavily vascularized; stamens (1 to)3; ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, usually free, close, stigmas 2. Caryopsis with the hilum short or long-linear (*Molinia*); endosperm hard, without lipid, containing compound starch grains; embryo large or small (*Amphipogon*), epiblast absent, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins meeting or overlapping (*Hakonechloa*). Basic chromosome numbers: $x = 6, 9, 12$.

Foliar anatomy. Mesophyll nonradiate or rarely radiate (*Arundo, Amphipogon*), without an adaxial palisade layer, without fusoid cells, arm cells absent or present (*Phragmites*); Kranz anatomy absent; midrib simple; adaxial bulliform cells present.

Foliar micromorphology. Stomata with low dome-shaped or triangular subsidiary cells; bicellular microhairs present or less commonly absent, when present of panicoid-type except in *Amphipogon*, which has unique microhair morphology; papillae absent except in *Amphipogon*.

Photosynthetic pathway. C_3.

INCLUDED TRIBE (NOW IDENTICAL TO SUBFAMILY AND THUS REDUNDANT):

Arundineae Dumort., Obs. Gram. Belg.: 82. 1824. TYPE: *Arundo* L.

Notes. The traditional Arundinoideae were well known as a dustbin group (e.g., Clayton & Renvoize, 1986; Kellogg & Campbell, 1987). A number of studies indicated that this subfamily as traditionally circumscribed was polyphyletic (e.g., Barker et al., 1995; Clark et al., 1995), although some support for a monophyletic Arundinoideae (including Arundinoideae s. str., Danthonioideae, Aristidoideae, *Micraira*, and *Eriachne*) was found by Hsiao et al. (1999). The results of the combined analysis presented here suggest that a monophyletic core arundinoid group does exist, even though individual data sets do not strongly support the group. The exact generic membership of the subfamily remains to be determined; however, we include the following genera: *Amphipogon, Arundo, Dregeochloa, Hakonechloa, Molinia* (and *Moliniopsis* if recognized), and *Phragmites*. We provisionally place the crinipoid group (*Crinipes, Dichaetaria, Elytrophorus, Leptagrostis, Nematopoa, Piptophyllum, Styppeiochloa,* and *Zenkeria*) here as well, based on molecular evidence from Linder et al. (1997) and Barker (1997). No morphological synapomorphies have been identified to support the monophyletic arundinoid clade, although Linder et al. (1997) linked *Arundo, Phragmites,* and *Molinia* by the presence of hollow culm internodes, a punctiform hilum, and convex adaxial rib sides in the leaf blade. This subfamily clearly requires further study. The subfamily includes 33 to 38 species, counting the crinipoids.

IX. Danthonioideae Barker & H. P. Linder, subfam. nov. TYPE: *Danthonia* DC. Fl. Franc. 3: 32. 1805. Figure 5F.

Haec subfamilia ab aliis subfamiliis Poacearum synergidis haustorialibus, ligula ciliata, embryone mesocotyledone praedito, spicula pluriflora vel si uni- vel biflora nunc rhachilla in extensionem desinente, stylorum basibus plerumque distantibus atque anatomia "Kranz" et micropilis chloridoideis carentibus bene distincta.

Distinct from the other subfamilies of the grasses by the haustorial synergids, and by the conjunction of a ciliate ligule, the presence of an embryo mesocotyl, a several-flowered spikelet, which, if 1- or 2-flowered, has a rachilla extension, usually distinctly separated style bases, the absence of Kranz anatomy, and the absence of chloridoid microhairs.

Plants perennial (caespitose, rhizomatous or stoloniferous) or less commonly annual, herbaceous or rarely suffrutescent, of mesic to xeric open habitats in grasslands, heathlands, and open woodlands. Culms solid or very rarely hollow. Leaves distichous; abaxial ligule usually absent (sometimes present in *Cortaderia, Karroochloa,* and *Pentaschistis*); adaxial ligule a fringe of hairs or a fringed membrane; blades relatively narrow, without a pseudopetiole, venation parallel; sheaths not auriculate except in *Pentameris thuarii*. Inflorescences paniculate or less commonly racemose or spicate, bracts outside of the spikelets absent (but the subtending leaf ± spatheate and disarticulating with the inflorescence in *Tribolium pusillum*). Spikelets bisexual (but sometimes without bisexual florets in *Cortaderia*) or unisexual (*Cortaderia, Lamprothyrsus*), glumes 2 and usually equal, female-fertile florets 1 to 6(to 20), with apical reduction and a rachilla extension usually present, laterally compressed, disarticulating above the glumes and between the florets, less commonly below the glumes; lemma lacking uncinate macrohairs, awn single and from a sinus; palea well developed, sometimes relatively short; lodicules 2, free (rarely joined), fleshy or rarely with an apical membranous flap, glabrous or ciliate, often with microhairs, sometimes heavily vascularized; stamens 3; ovary glabrous or rarely with apical hairs (*Pentameris*), apical appendage absent, haustorial synergids present, only weakly developed in a few taxa, styles 2, the bases usually widely separated, stigmas 2. Caryopsis with the hi-

lum short or long-linear; endosperm hard, containing compound starch grains (simple in *Prionanthium*); embryo large or small, epiblast absent, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins meeting (overlapping in *Danthonia decumbens*). Basic chromosome numbers: $x = 6, 7, 9$.

Foliar anatomy. Mesophyll nonradiate, an adaxial palisade layer absent, fusoid cells absent, arm cells absent; Kranz anatomy absent; midrib simple, usually with one bundle, an arc of bundles in *Cortaderia*; adaxial bulliform cells present or absent.

Foliar micromorphology. Stomata with dome-shaped or parallel-sided subsidiary cells (rarely high dome-shaped or slightly triangular); bicellular microhairs present, panicoid-type, sometimes absent; papillae usually absent but often present in *Chionochloa* and *Merxmuellera*.

Photosynthetic pathway. C_3.

INCLUDED TRIBE (NOW IDENTICAL TO SUBFAMILY AND THUS REDUNDANT):

Danthonieae Zotov, New Zealand J. Bot. 1 (1): 86. 1963. (Including Cortaderieae Zotov, New Zealand J. Bot. 1 (1): 83. 1963.) TYPE: *Danthonia* DC.

Notes. The presence of haustorial synergids in the ovule and distant styles support the monophyly of this clade (Verboom et al., 1994). Bilobed prophylls also may be a synapomorphy, but this feature has not been investigated sufficiently in the rest of the family. The results of this study indicate robust molecular support for the monophyly of this clade (excluding *Centropodia* and *Merxmuellera rangei*), but its placement within the larger PACCAD Clade is equivocal. Pending further studies of the diversity of the danthonioid grasses, we recognize only one tribe, which includes the following genera (sensu Barker et al., 2000): *Austrodanthonia, Chaetobromus, Chionochloa, Cortaderia, Danthonia, Joycea, Karroochloa, Lamprothyrsus, Merxmuellera* (minus *M. rangei*), *Notochloe, Notodanthonia, Pentameris, Pentaschistis, Plinthanthesis, Prionanthium, Pseudopentameris, Rytidosperma, Schismus,* and *Tribolium*. The subfamily includes approximately 250 species.

X. Centothecoideae Soderstr. [as "Centostecoideae"], Taxon 30: 615. 1981. TYPE: *Centotheca* Desv. Figure 5G.

Plants annual or perennial (rhizomatous or stoloniferous), herbaceous or reedlike, of warm temperate woodlands and tropical forests. Culms solid or hollow. Leaves distichous; abaxial ligule absent or present as a line of hairs (*Calderonella, Thysanolaena*); adaxial ligule membranous or ciliate, or membranous with ciliate margins; blades relatively broad to narrow, often pseudopetiolate, venation parallel; sheaths sometimes auriculate. Inflorescences racemose or paniculate, bracts outside of the spikelets absent. Spikelets bisexual or unisexual, (1 to)2- to many-flowered with reduction either above or below the fertile florets, often compressed laterally; lemma lacking uncinate macrohairs, if awned, the awn single; palea usually well developed, sometimes relatively short; lodicules 2 or absent, ± cuneate, many-nerved or less commonly not or scarcely vascularized; stamens (1 to)2 or 3; ovary glabrous, apical appendage absent, haustorial synergids presumed absent, styles 2, free or fused, close, stigmas 2. Caryopsis with the hilum basal, punctiform; endosperm hard, without lipid, containing simple or compound starch grains; embryo small or large, the epiblast present, scutellar cleft present, mesocotyl internode present, embryonic leaf margins overlapping. Basic chromosome number: $x = 12$ ($x = 11$ or 12? in *Thysanolaena*).

Foliar anatomy. Mesophyll nonradiate, often with an adaxial palisade layer, fusoid-like cells frequently present as extensions of the outer parenchyma bundle sheath, arm cells absent; Kranz anatomy absent; midrib simple; adaxial bulliform cells large.

Foliar micromorphology. Stomata with dome-shaped and/or triangular subsidiary cells; bicellular microhairs present, panicoid-type; papillae absent.

Photosynthetic pathway. C_3.

INCLUDED TRIBES:

Centotheceae Ridl., Mat. Fl. Malay Pen. 3: 122. 1907. TYPE: *Centotheca* P. Beauv.

Thysanolaeneae C. E. Hubb., in Hutch., Fam. Fl. Pl. 2: 222. 1934. TYPE: *Thysanolaena* Nees.

Notes. Support for the monophyly of this subfamily as recognized here is moderate, and no morphological synapomorphies have been identified. The sister relationship between the centothecoid and panicoid clades, however, is relatively robust. The positions of *Gynerium* and *Danthoniopsis* are unstable. A majority of the Centotheceae are characterized by unusual leaf anatomy, including the presence of palisade mesophyll and laterally extended bundle sheath cells. Additional study of this clade is under way (J. G. Sánchez-Ken, pers.

comm.). The subfamily includes approximately 45 species.

XI. Panicoideae Link, Hort. Berol. 1: 202. 1827. TYPE: *Panicum* L. Figures 5B, E, 6F, I, K.

Syn.: Andropogonoideae Burmeist., Handb. Naturgesch.: 201. 1837.
Rottboellioideae Burmeist., Handb. Naturgesch.: 202. 1837.
Saccharoideae (Rchb.) Horan., Char. Ess. Fam.: 34. 1847.

Plants annual or perennial (rhizomatous, stoloniferous, caespitose or decumbent), primarily herbaceous, of the tropics and subtropics, but also diverse in the temperate zone. Culms solid or less commonly hollow. Leaves distichous; abaxial ligule usually absent, occasionally present as a line of hairs; adaxial ligule a fringed or unfringed membrane, or a fringe of hairs, or sometimes absent; blades relatively broad to narrow, sometimes pseudopetiolate, venation parallel; sheaths usually nonauriculate. Inflorescences panicles, racemes, or spikes, or complex combinations of these, bracts outside of the spikelets present (Andropogoneae) or absent (Paniceae). Spikelets bisexual or unisexual (if the latter plants dioecious or monoecious), frequently paired in combinations with long and short pedicels, usually with glumes 2, sterile lemma 1, and female-fertile floret 1, dorsally compressed or less commonly not compressed or laterally compressed, disarticulating below the glumes (above the glumes in Arundinelleae) or the inflorescence axes breaking apart; lemma lacking uncinate macrohairs, if awned, the awn single; palea well developed (Paniceae) or reduced to absent (Andropogoneae); lodicules 2 or sometimes absent, cuneate, free, fleshy, usually glabrous; stamens 3; ovary usually glabrous, apical appendage absent, haustorial synergids absent, styles 2, free or fused, close, stigmas 2 (rarely 1 or 3). Caryopsis with the hilum usually short; endosperm hard, without lipid, containing simple or less commonly compound starch grains; embryo usually large, epiblast absent or rarely present, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins overlapping or rarely meeting. Basic chromosome numbers: $x = 5$, (7), 9, 10, (12), (14).

Foliar anatomy. Mesophyll radiate or nonradiate, an adaxial palisade layer absent, fusoid cells absent except in *Homolepis* and *Streptostachys*, arm cells usually absent; Kranz anatomy present or absent; midrib simple or rarely complex; adaxial bulliform cells present.

Foliar micromorphology. Stomata with triangular or dome-shaped subsidiary cells; bicellular microhairs present, panicoid-type, rarely absent; papillae absent or present (mostly in the Andropogoneae).

Photosynthetic pathway. C_3, C_4 (PCK, NAD-ME and NADP-ME), and some C_3/C_4 intermediates.

INCLUDED TRIBES:

Andropogoneae Dumort. [as "Andropogineae"], Observ. Gramin. Belg.: 84. 1824. TYPE: *Andropogon* L.
Arundinelleae Stapf, Fl. Cap. 7: 314. 1898. TYPE: *Arundinella* Raddi.
Hubbardieae C. E. Hubb., in Bor, Grasses India Burma Ceylon Pakistan: 685. 1960. TYPE: *Hubbardia* Bor.
Isachneae Benth., J. Linn. Soc. Bot. 19: 30. 1881. TYPE: *Isachne* R. Br.
Paniceae R. Br., Voy. Terra Austr. 2: 582. 1814. TYPE: *Panicum* L.
Steyermarkochloeae Davidse & R. P. Ellis, Ann. Missouri Bot. Gard. 71: 994. 1984. TYPE: *Steyermarkochloa* Davidse & R. P. Ellis.

Notes. While support for the panicoid/centothecoid clade is high, relationships within the clade remain unclear. No robust phylogeny for the Panicoideae is yet available, although work is in progress (Giussani et al., in press; Duvall et al., in press). Preliminary results indicate that the Paniceae as currently circumscribed may not be monophyletic, and that the large genus *Panicum* is polyphyletic (Zuloaga et al., 2000; Gómez-Martinez & Culham, 2000). Andropogoneae + *Arundinella* appear to be monophyletic (Spangler et al., 1999); other genera of the Arundinelleae are likely to be distributed among the Andropogoneae, Paniceae, and perhaps even the Centothecoideae (Kellogg, 2000b). This subfamily includes approximately 3270 species.

XII. Chloridoideae Kunth ex Beilschm., Flora 16(2): 52, 105. 1833. TYPE: *Chloris* Sw. Figures 5D, H, 6E, M, N, 7C.

Syn.: Pappophoroideae Burmeist., Handb. Naturgesch. 205. 1837.
Eragrostoideae Pilger, Nat. Pfl.-Fam. ed. 2, 14d: 167. 1956.

Plants annual or perennial (rhizomatous, stoloniferous, caespitose or decumbent), herbaceous (rarely woody), of dry climates, especially in the tropics and subtropics, also found in the temperate zone. Culms solid or hollow. Leaves distichous; abaxial ligule usually absent, rarely present as a line of hairs; adaxial ligule a fringed or less commonly

unfringed membrane; blades relatively narrow, without pseudopetioles, venation parallel; sheaths usually non-auriculate. Inflorescences paniculate, paniculate with spicate branches, racemose, or spicate, bracts outside of the spikelets absent. Spikelets bisexual or sometimes unisexual (if so the plants dioecious or monoecious), with glumes 2, rarely a sterile lemma, and female-fertile florets 1 to many, apical reduction usually present, usually laterally compressed, sometimes dorsally compressed, usually disarticulating above the glumes (below in a few *Eragrostis* species); lemma lacking uncinate macrohairs, if awned, the awns single or if multiple, lacking a basal column; palea well developed; lodicules 2 or absent, fleshy, glabrous; stamens 1 to 3; ovary glabrous, apical appendage absent, haustorial synergids absent, styles 2, free, close, stigmas 2. Caryopsis with the pericarp often free or loose; hilum short; endosperm hard, without lipid, containing simple or compound starch grains; embryo large or rarely small, epiblast present or rarely absent, scutellar cleft present, mesocotyl internode elongated, embryonic leaf margins meeting or rarely overlapping. Basic chromosome numbers: $x = (7), (8), 9, 10$.

Foliar anatomy. Mesophyll usually radiate, without an adaxial palisade layer, fusoid cells absent, arm cells absent; Kranz anatomy present; midrib simple; adaxial bulliform cells present.

Foliar micromorphology. Stomata with dome-shaped or triangular subsidiary cells; bicellular microhairs present, usually chloridoid-type; papillae absent or present.

Photosynthetic pathway. C_3 (*Eragrostis walteri, Merxmuellera rangei*), otherwise C_4 (PCK, NAD-ME, but reported as NADP-ME in *Pappophorum*, by Hattersley & Watson, 1992; the latter may be an error).

INCLUDED TRIBES:

Cynodonteae Dumort., Observ. Gramin. Belg.: 83. 1824. TYPE: *Cynodon* Rich.

Eragrostideae Stapf, Fl. Cap. 7: 316. 1898. TYPE: *Eragrostis* Wolf.

Leptureae Dumort., Observ. Gramin. Belg.: 83. 1824. TYPE: *Lepturus* R. Br.

Orcuttieae Reeder, Madroño 18: 20. 1965. TYPE: *Orcuttia* Vasey.

Pappophoreae Kunth, Rév. Gramin. 1: 82. 1829. TYPE: *Pappophorum* Schreb.

INCERTAE SEDIS:

Centropodia Reichenb., *Merxmuellera rangei* (Pilg.) Conert

Notes. Reduction in the number of veins in the lemma is a general trend within the subfamily but is clearly not a synapomorphy. Except for the C_3 *Eragrostis walteri* and *Merxmuellera rangei*, the Chloridoideae are uniformly C_4 with both the NAD-ME and PCK subtypes. The current tribal classification for this subfamily conflicts with molecular data and is likely to be modified (Hilu et al., 1999). This subfamily includes approximately 1400 species.

XIII. Incertae Sedis

Eriachneae (Ohwi) Eck-Borsb., Blumea 26: 128. 1980.

Micraireae Pilger, Nat. Pfl.-Fam. Ed. 2, 14d: 167. 1956.

Streptogyneae C. Calderón & Soderstr., Smithsonian Contr. Bot. 44: 18. 1980.

Cyperochloa Lazarides & L. Watson, Brunonia 9: 216. 1987.

Gynerium Willd. ex P. Beauv., Ess. Agrostogr. 138, 153, t. 24. 1812.

Notes. These five taxa are left Incertae Sedis because the data presented here do not firmly support their inclusion in any of the 12 subfamilies. This approach has also been taken by the APG (1998) for taxa of uncertain placement. Some possible placements of the five taxa above will require publication of new names, and we feel strongly that nomenclatural changes should not be made until appreciable data support the conclusion. That said, recent unpublished data (J. G. Sánchez-Ken, pers. comm.) suggest that *Gynerium* can be placed as its own tribe in Panicoideae, and the tribal name may be available by the time this paper is published (Sánchez-Ken & Clark, 2001). It is likely that *Cyperochloa* will be placed in Centothecoideae, but this is based on its morphological similarities to *Spartochloa* and not on any data on *Cyperochloa* itself. Streptogyneae will probably fall within Ehrhartoideae, but limitations of our data and lack of support in our trees make us cautious about placing it there unequivocally; there may be an argument for recognition of the tribe as its own subfamily. The name Micrairoideae has been published (Pilger, 1956). Our data are too limited and the placement of the group too uncertain to add it as a thirteenth subfamily, although flora writers may choose to do so. Our data on *Eriachne* are weak, and show only that the genus does not fall within the Pani-

coideae, where it has been placed traditionally. Its placement near the base of the PACCAD Clade is based on a single-stranded *rbc*L sequence from one species, and an ITS sequence from a second. The two species represent two sections of the genus, one of which has actually been recognized as its own genus. We therefore feel that Incertae Sedis best reflects what we know of the position of the tribe—its position is uncertain.

Literature Cited

Ambrose, B. A., D. R. Lerner, P. Ciceri, C. M. Padilla, M. F. Yanofsky & R. J. Schmidt. 2000. Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. Molec. Cell 5: 569–579.

APG (Angiosperm Phylogeny Group). 1998. An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard. 85: 531–553.

Avdulov, N. P. 1931. Kario-sistematicheskoye issledovaniye semeystva zlakov. Trudy Prikl. Bot. Prilozheniye 44: 1–352. [Karyosystematic studies in the grass family. Supplement 44 to The Bull. Appl. Bot. Genet. Pl.-Breed., Leningrad. Russian text, pp. 1–352; German summary, pp. 353–425; index pp. 426–428. English translation (mislabeled as supplement 43) published by the Smithsonian Institution and the National Scientific Documentation Centre, New Delhi. 1975. TT 70-53085.]

Barker, N. P. 1995. A Molecular Phylogeny of the Subfamily Arundinoideae. Ph.D. Thesis, University of Cape Town.

———. 1997. The relationships of *Amphipogon*, *Elytrophorus*, and *Cyperochloa* (Poaceae) as suggested by *rbc*L sequence data. Telopea 7: 205–213.

———, H. P. Linder & E. H. Harley. 1995. Polyphyly of Arundinoideae (Poaceae): Evidence from *rbc*L sequence data. Syst. Bot. 20: 423–435.

———, ——— & ———. 1999. Sequences of the grass-specific insert in the chloroplast *rpo*C2 gene elucidate generic relationships of the Arundinoideae (Poaceae). Syst. Bot. 23: 327–350.

———, C. M. Morton & H. P. Linder. 2000. The Danthonieae: Generic composition and relationships. Pp. 221–229 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria.

Barkworth, M. E. 1993. North American Stipeae: Taxonomic notes and other comments. Phytologia 74: 1–25.

——— & J. Everett. 1987. Evolution in the Stipeae: Identification and relationships of its monophyletic taxa. Pp. 251–264 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

Baum, D. A., K. J. Sytsma & P. C. Hoch. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. Syst. Bot. 19: 363–388.

———, R. L. Small & J. F. Wendel. 1998. Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. Syst. Biol. 47: 181–207.

Bennetzen, J. L. & E. A. Kellogg. 1997. Do plants have a one-way ticket to genomic obesity? Pl. Cell 9: 1509–1514.

Bentham, G. 1878. Flora Australiensis 7: 449–670.

——— & J. D. Hooker. 1883. Gramineae. Pp. 1074–1215 *in* Genera Plantarum, vol. 3, pt. 2. L. Reeve, London.

Borre, A. Van den. 1994. Taxonomy of the Chloridoideae (Poaceae), with Special Reference to the Genus *Eragrostis*. Unpublished Ph.D. Dissertation, Australian National University, Canberra.

——— & L. Watson. 1994. The infrageneric classification of *Eragrostis* (Poaceae). Taxon 43: 383–422.

Bossinger, G. 1990. Klassifizierung von Entwicklungsmutanten der Gerste anhand einer Interpretation des Pflanzenaufbaus der Poaceae aus Phytomeren. Inaugural-Dissertation, Rheinischen Friedrich-Wilhelms-Universität zu Bonn.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

———. 1990. Combinable component consensus. Cladistics 6: 369–372.

Briggs, B. G., A. D. Marchant, S. Gilmore & C. L. Porter. 2000. A molecular phylogeny of Restionaceae and allies. Pp. 661–671 *in* K. L. Wilson & D. A. Morrison (editors), Monocots: Systematics and Evolution. CSIRO Press, Sydney.

Brown, R. 1810. Prodromus florae Novae Hollandiae et insulae Van-Diemen, vol. 1. J. Johnson, London.

———. 1814. General remarks, geographical and systematical, on the botany of Terra Australis, Appendix 3. Pp. 580–583 *in* M. Flinders (editor), A Voyage to Terra Australis; Undertaken for the Purpose of Completing the Discovery of that Vast Country, and Prosecuted in the Years 1801, 1802, and 1802. W. Bulmer, London.

Brown, W. V. 1958. Leaf anatomy in grass systematics. Bot. Gaz. (Crawfordsville) 119: 170–178.

———. 1977. The Kranz syndrome and its subtypes in grass systematics. Mem. Torrey Bot. Club 23: 1–91.

Calderón, C. E. & T. R. Soderstrom. 1980. The genera of Bambusoideae (Poaceae) of the American continent: Key and comments. Smithsonian Contr. Bot. 44: 1–27.

Campbell, C. S. 1985. The subfamilies and tribes of Gramineae (Poaceae) in the southeastern United States. J. Arnold Arbor. 66: 123–199.

——— & E. A. Kellogg. 1987. Sister group relationships of the Poaceae. Pp. 217–224 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

———, P. E. Garwood & L. P. Specht. 1986. Bambusoid affinities of the North American temperate genus *Brachyelytrum* (Gramineae). Bull. Torrey Bot. Club 113: 135–141.

Caro, J. A. 1982. Sinopsis taxonómica de las gramíneas argentinas. Dominguezia 4: 1–51.

Carolin, R. C., S. W. L. Jacobs & M. Vesk. 1973. The structure of the cells of the mesophyll and parenchymatous bundle sheath of the Gramineae. Bot. J. Linn. Soc, London 66: 269–273.

Čelakovský, L. 1889. Über den Ärchenbau der Brasilianischen Grasgattung *Streptochaeta* Schrader. Sitzungsber. Königl. Böhm. Ges. Wiss. Prag, Math.-Naturwiss. Cl. 3: 14–42, table 2. [Unpublished English translation by Mrs. G. Saad, 1974, for the Smithsonian Institution and the National Science Foundation, Washington, D.C.; in

the files of the McClure Bamboo Library, Department of Botany, Smithsonian Institution, Washington, D.C.]
Chase, M. W., M. F. Fay & V. Savolainen. 2000a. Higher-level classification in the angiosperms: New insights from the perspective of DNA sequence data. Taxon 49: 685–704.
———, D. E. Soltis, P. S. Soltis, P. J. Rudall, M. F. Fay, W. J. Hahn, S. Sullivan, J. Joseph, M. Molvray, P. J. Kores, T. J. Givnish, K. J. Sytsma & J. C. Pires. 2000b. Higher-level systematics of the monocotyledons: An assessment of current knowledge and a new classification. Pp. 3–16 *in* K. L. Wilson & D. A. Morrison (editors), Monocots: Systematics and Evolution. CSIRO Press, Sydney.
———, ———, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, Y.-L. Qiu, K. A. Kron, J. H. Rettig, E. Conti, J. D. Palmer, J. R. Manhart, K. J. Sytsma, H. J. Michaels, W. J. Kress, K. G. Karol, W. D. Clark, M. Hedrén, B. S. Gaut, R. K. Jansen, K.-J. Kim, C. F. Wimpee, J. F. Smith, G. R. Furnier, S. H. Strauss, Q.-Y. Xiang, G. M. Plunkett, P. S. Soltis, S. M. Swensen, S. E. Williams, P. A. Gadek, C. J. Quinn, L. E. Eguiarte, E. Golenberg, G. H. Learn, Jr., S. W. Graham, S. C. H. Barrett, S. Dayanandan & V. A. Albert. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbc*L. Ann. Missouri Bot. Gard. 80: 528–580.
Chatterton, N. J., C. Hsiao, K. H. Asay, K. B. Jensen & R. R. Wang. 1992. Nucleotide sequence of the internal transcribed spacer region rDNA in barley, *Hordeum vulgare* L. (Gramineae). Pl. Molec. Biol. 20: 165–166.
Chen, Z., S. Muthukrishnan, G. H. Liang, K. F. Schertz & G. E. Hart. 1993. A chloroplast DNA deletion located in RNA polymerase gene *rpoC2* in CMS lines of sorghum. Molec. Gen. Genet. 236: 251–259.
Clark, L. G. 1991. The function of fusoid cells in bamboo: An hypothesis. Amer. J. Bot. 78 (suppl.): 22. [Abstract.]
——— & E. J. Judziewicz. 1996. The grass subfamilies Anomochlooideae and Pharoideae (Poaceae). Taxon 45: 641–645.
———, W. Zhang & J. F. Wendel. 1995. A phylogeny of the grass family (Poaceae) based on *ndh*F sequence data. Syst. Bot. 20: 436–460.
———, M. Kobayashi, S. Mathews, R. E. Spangler & E. A. Kellogg. 2000. The Puelioideae, a new subfamily of Poaceae. Syst. Bot. 25: 181–187.
Clayton, W. D. 1965. Introduction to Arber's "The Gramineae." Pp. i–xxxii *in* The Gramineae by A. Arber, 1965 reprint. J. Cramer, New York.
———. 1990. The spikelet. Pp. 32–51 *in* G. P. Chapman (editor), Reproductive Versatility in the Grasses. Cambridge Univ. Press, Cambridge, U.K.
——— & S. A. Renvoize. 1986. Genera Graminum, Grasses of the World. Her Majesty's Stationery Office, London.
Clifford, H. T. 1987. Spikelet and floral morphology. Pp. 21–30 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.
Crepet, W. L. & G. D. Feldman. 1991. The earliest remains of grasses in the fossil record. Amer. J. Bot. 78: 1010–1014.
Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia Univ. Press, New York.
Cummings, M. P., L. M. King & E. A. Kellogg. 1994. Slipped-strand mispairing in a plastid gene: *rpo*C2 in grasses (Poaceae). Molec. Biol. Evol. 11: 1–8.
Dahlgren, R. M. T., H. T. Clifford & P. F. Yeo. 1985. The Families of the Monocotyledons. Springer-Verlag, Berlin, Heidelberg, New York.
Davis, J. I. & R. J. Soreng. 1993. Phylogenetic structure in the grass family (Poaceae) as inferred from chloroplast DNA restriction site variation. Amer. J. Bot. 80: 1444–1454.
Dehesh, K., J. Tepperman, A. H. Christensen & P. H. Quail. 1991. *phy*B is evolutionarily conserved and constitutively expressed in rice seedling shoots. Molec. Gen. Genet. 225: 305–313.
Devos, K. M., M. D. Atkinson, C. N. Chinoy, H. A. Francis, R. L. Harcourt, R. M. D. Koebner, C. J. Liu, P. Masoj'c, D. X. Xie & M. D. Gale. 1993. Chromosomal rearrangements in the rye genome relative to that of wheat. Theor. Appl. Genet. 85: 673–680.
Doebley, J., M. Durbin, E. M. Golenberg, M. T. Clegg & D. P. Ma. 1990. Evolutionary analysis of the large subunit of carboxylase (*rbc*L) nucleotide sequence data among the grasses (Poaceae). Evolution 44: 1097–1108.
Donoghue, M. J., R. G. Olmstead, J. F. Smith & J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann. Missouri Bot. Gard. 79: 249–265.
Doyle, J. J., J. I. Davis, R. J. Soreng, D. Garvin & M. J. Anderson. 1992. Chloroplast DNA inversions and the origin of the grass family (Poaceae). Proc. Natl. Acad. Sci. U.S.A. 89: 7722–7726.
Duval-Jouve, M. J. 1875. Histotaxie des feuilles des Graminées. Ann. Sci. Nat. Bot. 8: 227–346.
Duvall, M. R. & B. R. Morton. 1996. Molecular phylogenetics of Poaceae: An expanded analysis of *rbc*L sequence data. Molec. Phylogenet. Evol. 5: 353–358.
———, M. T. Clegg, M. W. Chase, W. D. Clark, W. J. Kress, H. G. Hills, L. E. Eguiarte, J. F. Smith, B. S. Gaut, E. A. Zimmer & G. H. Learn, Jr. 1993. Phylogenetic hypotheses for the monocotyledons constructed from *rbc*L sequence data. Ann. Missouri Bot. Gard. 80: 607–619.
———, J. D. Noll & A. H. Minn. In press. Phylogenetics of Paniceae (Poaceae). Amer. J. Bot.
Ellis, R. P. 1981. Leaf anatomy of the South African Danthonieae (Poaceae). V. *Merxmuellera macowanii*, *M. davyi* and *M. aureocephala*. Bothalia 13: 493–500.
Engler, A. 1892. Syllabus der Vorlesungen über specielle und Medicinisch-pharmaceutisch Botanik. Gebrüder Borntrager, Berlin.
Esen, A. & K. W. Hilu. 1989. Immunological affinities among subfamilies of the Poaceae. Amer. J. Bot. 76: 196–203.
Farris, J. S., M. Källersjö, A. G. Kluge & C. Bult. 1994. Testing significance of incongruence. Cladistics 10: 315–319.
Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.
Fisher, J. B. & J. Dransfield. 1977. Comparative morphology and development of inflorescence adnation in rattan palms. Bot. J. Linn. Soc. 75: 119–140.
Frazier, P. J., P. Richmond & A. M. Donald (editors). 1997. Starch: Structure and Functionality. Royal Society of Chemistry, Information Services, Cambridge, U.K.
Gale, M. D. & K. M. Devos. 1998. Comparative genetics in the grasses. Proc. Natl. Acad. Sci. U.S.A. 95: 1971–1974.

Gaut, B. S. & J. F. Doebley. 1997. DNA sequence evidence for the segmental allotetraploid origin of maize. Proc. Natl. Acad. Sci. U.S.A. 94: 6809–6814.

———, S. V. Muse, W. Clark & M. T. Clegg. 1992. Relative rates of nucleotide substitution at the *rbc*L locus in monocotyledonous plants. J. Molec. Evol. 35: 292–303.

———, B. R. Morton, B. McCaig & M. T. Clegg. 1996. Substitution rate comparisons between grasses and palms: Synonymous rate differences at the nuclear gene *Adh* parallel rate differences at the plastid gene *rbc*L. Proc. Natl. Acad. Sci. U.S.A. 93: 10274–10279.

———, L. G. Clark, J. F. Wendel & S. V. Muse. 1997. Comparisons of the molecular evolutionary process at *rbc*L and *ndh*F in the grass family (Poaceae). Molec. Biol. Evol. 14: 769–777.

Ghopal, B. H. & H. Y. Mohan Ram. 1985. Systematic significance of mature embryo of bamboo. Pl. Syst. Evol. 148: 239–246.

Giussani, L. M., J. H. Cota-Sánchez, F. O. Zuloaga & E. A. Kellogg. In press. A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C_4 photosynthesis. Amer. J. Bot.

Goebel, K. 1895. Ein Beitrag zur Morphologie der Gräser. Flora 81: 17–19.

Goloboff, P. 1993. Nona, version 1.16. Buenos Aires: Distributed by the author.

Gómez-Martinez, R. & A. Culham. 2000. Phylogeny of the subfamily Panicoideae with emphasis on the tribe Paniceae: Evidence from the chloroplast *trn*L-F cpDNA region. Pp. 136–140 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria.

Gould, F. W. & R. B. Shaw. 1983. Grass Systematics, 2nd ed. Texas A&M Univ. Press, College Station.

GPWG (Grass Phylogeny Working Group). 2000. A phylogeny of the grass family (Poaceae), as inferred from eight character sets. Pp. 3–7 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria.

Grassl, C. O. 1956. The morphology of the grass spikelet with special reference to *Saccharum*. Proc. Int. Soc. Sugar Technologists 1: 764–780.

Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47: 9–17.

Greuter, W., J. McNeill, F. R. Barrie, H. M. Burdet, V. Demoulin, T. S. Filgueiras, D. H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland & D. L. Hawksworth (editors). 2000. International Code of Botanical Nomenclature (Saint Louis Code). Regnum Veg. 138.

Guédès, M. & P. Dupuy. 1976. Comparative morphology of lodicules in grasses. Bot. J. Linn. Soc. 73: 317–331.

Hackel, E. 1887. Gramineae. Pp. 1–97 *in* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien. Engelmann, Leipzig.

Hamby, R. K. & E. A. Zimmer. 1988. Ribosomal RNA sequences for inferring phylogeny within the grass family (Poaceae). Pl. Syst. Evol. 160: 29–37.

Hattersley, P. W. & L. Watson. 1992. Diversification of photosynthesis. Pp. 38–116 *in* G. P. Chapman (editor), Grass Domestication and Evolution. Cambridge Univ. Press, Cambridge, U.K.

Hillis, D. M. 1996. Inferring complex phylogenies. Nature 383: 130.

———. 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. Syst. Biol. 47: 3–8.

Hilu, K. W. & A. Esen. 1988. Prolamin size diversity in the Poaceae. Biochem. Syst. Ecol. 16: 457–465.

——— & J. L. Johnson. 1991. Chloroplast DNA reassociation and grass phylogeny. Pl. Syst. Evol. 176: 21–33.

——— & K. Wright. 1982. Systematics of Gramineae: A cluster analysis study. Taxon 31: 9–36.

———, L. A. Alice & H. Liang. 1999. Phylogeny of Poaceae inferred from *mat*K sequences. Ann. Missouri Bot. Gard. 86: 835–851.

Hiratsuka, J., H. Shimada, R. Whittier, T. Ishibashi, M. Sakamoto, M. Mori, C. Kondo, Y. Honji, C. R. Sun, B. Y. Meng, Y. Q. Li, A. Kanno, Y. Nishizawa, A. Hirai, K. Shinozaki & M. Sugiura. 1989. The complete sequence of the rice (*Oryza sativa*) chloroplast genome: Intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. Molec. Gen. Genet. 217: 185–194.

Hitchcock, A. 1935. Manual of Grasses of the United States. U.S.D.A. Misc. Publ. 200.

——— & A. Chase. 1950. Manual of Grasses of the United States, 2nd ed. U.S.D.A. Misc. Publ. 200.

Hsiao, C., S. W. L. Jacobs, N. P. Barker & N. J. Chatterton. 1998. A molecular phylogeny of the subfamily Arundinoideae (Poaceae) based on sequences of rDNA. Austral. Syst. Bot. 11: 41–52.

———, ———, N. J. Chatterton & K. H. Asay. 1999. A molecular phylogeny of the grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). Austral. Syst. Bot. 11: 667–688.

Igloi, G. L., A. Meinke, I. Dory & H. Kossel. 1990. Nucleotide sequence of the maize chloroplast *rpo B/C1/C2* operon: Comparison between the derived protein primary structures from various organisms with respect to functional domains. Molec. Gen. Genet. 221: 379–394.

Irish, E. E. 1998. Grass spikelets: A thorny problem. BioEssays 20: 789–793.

Jacobs, B. F., J. D. Kingston & L. L. Jacobs. 1999. The origin of grass-dominated ecosystems. Ann. Missouri Bot. Gard. 86: 590–643.

Jacobs, S. W. L. 1987. Systematics of the chloridoid grasses. Pp. 277–286 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

——— & J. Everett. 1997. *Jarava plumosa* (Gramineae), a new combination for the species formerly known as *Stipa papposa*. Telopea 7: 301–302.

———, ———, M. E. Barkworth & C. Hsiao. 2000. Relationships within the stipoid grasses (Gramineae). Pp. 75–82 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO, Melbourne.

Jacques-Félix, H. 1962. Les graminées (Poaceae) d'Afrique tropicale, 1: Généralités, classification, description de genres. Bull. Agron. 246: 1–4.

Jirásek, V. 1968. Morphologie der Schuppchen (Lodiculae) von Grasern und ihre Terminologie, ein weiterer Beitrag zur Kenntnis des Baues der Lodiculae. Acta Univ. Carol., Biol. 1968: 321–344.

——— & M. Jozífová. 1968. Morphology of lodicules, their variability and importance in the taxonomy of the Poaceae family. Bol. Soc. Argent. Bot. 12: 324–349.

Johnston, C. R. & L. Watson. 1976. Microhairs: A universal characteristic of non-festucoid grass genera? Phytomorphology 26: 297–301.

Judziewicz, E. J. 1987. Taxonomy and Morphology of the Tribe Phareae (Poaceae: Bambusoideae). Unpublished Ph.D. Dissertation, University of Wisconsin, Madison.
——— & T. R. Soderstrom. 1989. Morphological, anatomical, and taxonomic studies in *Anomochloa* and *Streptochaeta* (Poaceae: Bambusoideae). Smithsonian Contr. Bot. 68.
———, L. G. Clark, X. Londoño & M. Stern. 1999. American Bamboos. Smithsonian Institution Press, Washington, D.C.
Källersjö, M., S. J. Farris, A. G. Kluge & C. Bult. 1992. Skewness and permutation. Cladistics 8: 275–287.
Kanai, R. & G. E. Edwards. 1999. The biochemistry of C_4 photosynthesis. Pp. 49–87 *in* R. F. Sage & R. K. Monson (editors), C_4 Plant Biology. Academic Press, San Diego.
Katayama, H. & Y. Ogihara. 1996. Phylogenetic affinities of the grasses to other monocots as revealed by molecular analysis of chloroplast DNA. Curr. Genet. 29: 572–581.
Kellogg, E. A. 1998. Relationships of cereal crops and other grasses. Proc. Natl. Acad. Sci. U.S.A. 95: 2005–2010.
———. 2000a. The grasses: A case study in macroevolution. Ann. Rev. Ecol. Syst. 31: 217–238.
———. 2000b. Molecular and morphological evolution in Andropogoneae. Pp. 149–158 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO, Melbourne.
——— & C. S. Campbell. 1987. Phylogenetic analyses of the Gramineae. Pp. 310–322 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.
——— & H. P. Linder. 1995. Phylogeny of Poales. Pp. 511–542 *in* P. J. Rudall, P. J. Cribb, D. F. Cutler & C. J. Humphries (editors), Monocotyledons: Systematics and Evolution. Royal Botanic Gardens, Kew, England.
Kennard, W., R. Phillips, R. Porter & A. Grombacher. 1999. A comparative map of wild rice (*Zizania palustris* L. $2n = 2x = 30$). Theor. Appl. Genet. 99: 793–799.
Kingston, J. D., B. D. Marino & A. Hill. 1994. Isotopic evidence for Neogene hominid paleoenvironments in the Kenya Rift Valley. Science 264: 955–959.
Klösgen, R. B., A. Gierl, Z. S. Schwarz-Sommer & H. Saedler. 1986. Molecular analysis of the waxy locus of *Zea mays*. Molec. Gen. Genet. 203: 237–244.
Latorre, C., J. Quade & W. C. McIntosh. 1997. The expansion of C_4 grasses and global change in the late Miocene: Stable isotope evidence from the Americas. Earth Planet. Sci. Lett. 146: 83–96.
Lazarides, M. 1995. The genus *Eriachne* (Eriachneae, Poaceae). Austral. Syst. Bot. 8: 355–452.
LeRoux, L. G. & E. A. Kellogg. 1999. Floral development and the formation of unisexual spikelets in the Andropogoneae (Poaceae). Amer. J. Bot. 86: 354–366.
Liang, H. & K. W. Hilu. 1996. Application of the *mat*K gene sequences to grass systematics. Canad. J. Bot. 74: 125–134.
Liese, W. 1998. The Anatomy of Bamboo Culms. Technical Report 18. International Network for Bamboo and Rattan, Beijing.
Linder, H. P. 1987. The evolutionary history of the Poales/Restionales—A hypothesis. Kew Bull. 42: 297–318.
———. 1992a. The gynoecia of Australian Restionaceae: Morphology, anatomy and systematic implications. Austral. Syst. Bot. 5: 227–245.
———. 1992b. The structure and evolution of the female flower of the African Restionaceae. Bot. J. Linn. Soc. 109: 401–425.
——— & G. Davidse. 1997. The systematics of *Tribolium* Desv. (Danthonieae, Poaceae). Bot. Jarhb. Syst. 119: 445–507.
——— & I. K. Ferguson. 1985. On the pollen morphology and phylogeny of the Restionales and Poales. Grana 24: 65–76.
——— & G. A. Verboom. 1996. Generic limits in the *Rytidosperma* (Danthonieae, Poaceae) complex. Telopea 6: 597–627.
———, ——— & N. P. Barker. 1997. Phylogeny and evolution in the *Crinipes* group of grasses (*Arundinoideae: Poaceae*). Kew Bull. 52: 91–110.
Lou, H., B. Zhang & D.-F. Qi. 1989. M13 cloning and nucleotide sequence of the sorghum chloroplast gene for the large subunit of ribulose-1,5-bisphosphate carboxylase. Acta Biochim. Biophys. Sin. 21: 179–188.
Maddison, W. P. & D. R. Maddison. 1993. MacClade, version 3. Analysis of Phylogeny and Character Evolution. Sinauer, Sunderland, Massachusetts.
Mason-Gamer, R. J. & E. A. Kellogg. 1996. Testing for phylogenetic conflict among molecular data sets in the Triticeae. Syst. Biol. 45: 524–545.
———, C. F. Weil & E. A. Kellogg. 1998. Granule-bound starch synthase: Structure, function, and phylogenetic utility. Molec. Biol. Evol. 15: 1658–1673.
Mathews, S. & R. A. Sharrock. 1996. The phytochrome gene family in grasses (Poaceae): A phylogeny and evidence that grasses have a subset of the loci found in dicot angiosperms. Molec. Biol. Evol. 13: 1141–1150.
———, M. Lavin & R. A. Sharrock. 1995. Evolution of the phytochrome gene family and its utility for phylogenetic analyses of angiosperms. Ann. Missouri Bot. Gard. 82: 296–321.
———, R. C. Tsai & E. A. Kellogg. 2000. Phylogenetic structure in the grass family (Poaceae): Evidence from the nuclear gene phytochrome B. Amer. J. Bot. 87: 96–107.
McClure, F. A. 1966. The Bamboos: A Fresh Perspective. Harvard Univ. Press, Cambridge, Massachusetts.
———. 1973. Genera of bamboos native to the New World (Gramineae: Bambusoideae). Smithsonian Contr. Bot. 9: 1–148.
Metcalfe, C. R. 1960. Anatomy of the Monocotyledons. I. Gramineae. Clarendon Press, Oxford.
Moore, G., K. M. Devos, Z. Wang & M. D. Gale. 1995. Grasses, line up and form a circle. Curr. Biol. 5: 737–739.
Morales, C. O. 1998. Nomenklatorische Probleme in Bezug auf die Namen Phareae und Leptaspideae (Gramineae). Sendtnera 5: 243.
Nadot, S., R. Bajon & B. Lejeune. 1994. The chloroplast gene *rps*4 as a tool for the study of Poaceae phylogeny. Pl. Syst. Evol. 191: 27–38.
Nakai, T. 1943. Anomochloaceae. *In* Ordines, Familiae, Tribi, Genera, a Prof. Nakai, Edita 222.
Nambudiri, E. M. V., W. D. Tidwell, B. N. Smith & N. P. Hebbert. 1978. A C_4 plant from the Pliocene. Nature 276: 816–817.
Nishizawa, Y. & A. Hirai. 1987. Nucleotide sequence and expression of the gene for the large subunit of rice ribulose 1, 5-bisphosphate carboxylase. Jap. J. Genet. 62: 389–395.
Nixon, K. C. 1993. Clados, version 1.4.98 (computer software and manual). Distributed by the author.

———. 2000. Winclada, version 0.9.99m6.1 (computer software and manual). Distributed by the author.

Núñez, O. 1968. El problema de la pálea de *Oryza* L. Bol. Soc. Argent. Bot. 12: 57–97.

Pilger, R. 1956. Gramineae II, Unterfam. Micrairoideae. P. 167 *in* A. Engler & K. Prantl, Die natürlichen Pflanzenfamilien (ed. 2) Band 14d. Duncker & Humblot, Berlin.

Poe, S. & D. L. Swofford. 1999. Taxon sampling revisited. Nature 398: 299–300.

Pohl, R. W. 1987. Man and the grasses: A history. Pp. 355–358 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

Pozzi, C., P. Faccioli, V. Terzi, A. M. Stanca, S. Cerioli, P. Castiglioni, R. Fink, R. Capone, K. J. Müller, G. Bossinger, W. Rohde & F. Salamini. 2000. Genetics of mutations affecting the development of a barley floral bract. Genetics 154: 1335–1346.

Prat, H. 1932. L'epiderme des graminées: étude anatomique et systematique. Ann. Sci. Nat. Bot. 14: 117–324.

———. 1936. La systématique des Graminées. Ann. Sci. Nat. Bot., ser. 10, 18: 165–258.

———. 1960. Vers une classification naturelle des graminées. Bull. Soc. Bot. France 107: 32–79.

Reeder, J. R. 1953. The embryo of *Streptochaeta* and its bearing on the homology of the coleoptile. Amer. J. Bot. 49: 77–80.

———. 1957. The embryo in grass systematics. Amer. J. Bot. 44: 756–769.

———. 1961. The grass embryo in systematics. Pp. 91–96 *in* Recent Advances in Botany, Vol. 1. Univ. Toronto Press, Toronto.

———. 1962. The Bambusoid embryo: A reappraisal. Amer. J. Bot. 49: 639–641.

Renner, S. S., D. B. Foreman & D. Murray. 2000. Timing transantarctic disjunctions in the Atherospermataceae (Laurales): Evidence from coding and noncoding chloroplast sequences. Syst. Biol. 49: 579–591.

Renvoize, S. A. & W. D. Clayton. 1992. Classification and evolution of the grasses. Pp. 3–37 *in* G. P. Chapman (editor), Grass Evolution and Domestication. Cambridge Univ. Press, Cambridge, U.K.

Rohde, W., D. Becker & F. Salamini. 1988. Structural analysis of the waxy locus from *Hordeum vulgare*. Nucl. Acids Res. 16: 7185–7186.

Rosengurtt, B., A. Laguardia & B. R. Arrillaga de Maffei. 1972. El caracter lípido del endosperma central en especies de Gramíneas. Bol. Fac. Agron. Univ. Montevideo 124: 1–43.

Roshevits, R. Yu. 1937. Zlaki: Vvedenie v Izuchenie Kormovykh Zlakov. [Grasses: An introduction to the study of fodder and cereal grasses.] Sel'khozgiz, Gosudarstvennoe Izdatel'stvo Kolkhoznoi I Sovkhoznoi Literatury. Agricultural Publishing House, Moscow, Leningrad. [English translation published for the Smithsonian Institution and the National Science Foundation, Washington, D.C., by the Indian National Scientific Documentation Centre, New Delhi. 1980. TT 72-51033.]

———. 1946. Sistema zlakov v svyazi s ikh evolyutsiei. Kamarov Botanical Institute, U.S.S.R. Academy of Sciences, Leningrad. [Portuguese translation by T. Sendulsky. 1969. Evolução e sistemática das gramíneas. Bol. Inst. Bot. (São Paulo) 5: 1–20.]

Sage, R. F. & R. K. Monson. 1999. C_4 Plant Biology. Academic Press, San Diego.

Sánchez-Ken, J. G. & L. G. Clark. 2001. Gynerieae, a new Neotropical tribe of grasses (Poaceae). Novon 11: 350–352.

Schmidt, R. J. & B. A. Ambrose. 1998. The blooming of grass flower development. Curr. Opinion Pl. Biol. 1: 60–67.

Schuster, J. 1910. Über die Morphologie der Grasblüte. Flora 100: 213–266, plates 2–5.

Seberg, O. & I. Linde-Laursen. 1996. *Eremium*, a new genus of the Triticeae (Poaceae) from Argentina. Syst. Bot. 21: 3–15.

Sendulsky, T., T. S. Filgueiras & A. G. Burman. 1987. Fruits, embryos, and seedlings. Pp. 31–36 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

Serebryakova, T. I. 1971. Morfogenez pobegov i evoluiutsiia zhiznennykh form zlakov [Shoot Morphogenesis and Evolution of Life Forms in Grasses]. Izdatel'stvo "Nauka," Moskva.

Shantz, H. L. 1954. The place of grasslands in the earth's cover of vegetation. Ecology 35: 143–145.

Simon, B. K. & S. W. L. Jacobs. 1990. Gondwanan grasses in the Australian flora. Austrobaileya 3: 239–260.

Sinha, N. R. & E. A. Kellogg. 1996. Parallelism and diversity in multiple origins of C_4 photosynthesis in the grass family. Amer. J. Bot. 83: 1458–1470.

Soderstrom, T. R. 1967. Taxonomic study of subgenus *Podosemum* and section *Epicampes* of *Muhlenbergia* (Gramineae). Contr. U.S. Natl. Herb. 34: 75–189 + 14 pl.

———. 1981. The grass subfamily Centostecoideae. Taxon 30: 614–616.

——— & R. P. Ellis. 1987. The position of bamboo genera and allies in a system of grass classification. Pp. 225–238 *in* T. R. Soderstrom, K. W. Hilu, C. S. Campbell & M. E. Barkworth (editors), Grass Systematics and Evolution. Smithsonian Institution Press, Washington, D.C.

———, ——— & E. J. Judziewicz. 1987. The Phareae and Streptogyneae of Sri Lanka: A morphological-anatomical study. Smithsonian Contr. Bot. 65.

Soltis, D. E., P. S. Soltis, M. E. Mort, M. W. Chase, V. Savolainen, S. B. Hoot & C. M. Morton. 1998. Inferring complex phylogenies using parsimony: An empirical approach using three large DNA data sets for angiosperms. Syst. Biol. 47: 32–42.

Soreng, R. J. & J. I. Davis. 1998. Phylogenetics and character evolution in the grass family (Poaceae): Simultaneous analysis of morphological and chloroplast DNA restriction site character sets. Bot. Rev. 64: 1–85.

——— & ———. 2000. Phylogenetic structure in Poaceae subfamily Pooideae as inferred from molecular and morphological characters: Misclassification vs. reticulation. Pp. 61–74 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria.

———, ——— & J. J. Doyle. 1990. A phylogenetic analysis of chloroplast DNA restriction site variation in Poaceae subfam. Pooideae. Pl. Syst. Evol. 172: 83–97.

Spangler, R., B. Zaitchik, E. Russo & E. A. Kellogg. 1999. Andropogoneae evolution and generic limits in *Sorghum* (Poaceae) using *ndh*F sequences. Syst. Bot. 24: 267–281.

Stapleton, C. M. A. 1997. The morphology of woody bam-

boos. Pp. 251–267 *in* G. P. Chapman (editor), The Bamboos, Academic Press, London.

Stebbins, G. L. 1974. Flowering Plants: Evolution above the Species Level. Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.

——— & B. Crampton. 1961. A suggested revision of the grass genera of temperate North America. Pp. 133–145 *in* Recent Advances in Botany, Vol. 1. Univ. Toronto Press, Toronto.

Sugiura, M. 1989. *Oryza sativa* chloroplast DNA 134,525 bp. Nagoya University, Center for Gene Research, Nagoya, Japan.

Swofford, D. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sinauer, Sunderland, Massachusetts.

Takaiwa, F., K. Oono, Y. Iida & M. Sugiura. 1985. The complete nucleotide sequence of a rice 25S rRNA gene. Gene 37: 255–259.

Tateoka, T. 1957. Miscellaneous papers on the phylogeny of the Poaceae (10). Proposition of a new phylogenetic system of Poaceae. J. Jap. Bot. 32: 275–287.

———. 1962. Starch grains of endosperm in grass systematics. Bot. Mag. (Tokyo) 75: 336–343.

———, S. Inoue & S. Kawano. 1959. Notes on some grasses. IX. Systematic significance of bicellular microhairs of leaf epidermis. Bot. Gaz. 121: 80–91.

Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37: 221–244.

Terrell, E. E. 1971. Survey of occurrences of liquid or soft endosperm in grass genera. Bull. Torrey Bot. Club 98: 264–268.

Thompson, J. D., D. G. Higgins & T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22: 4673–4680.

Tieghem, Ph. van. 1897. Morphologie de l'embryon et de la plantule chez les Graminées et les Cypéracées. Ann. Sci. Nat. Bot. 3: 259–309.

Tzvelev, N. N. 1989. The system of grasses (Poaceae) and their evolution. Bot. Rev. 55: 141–203.

Verboom, G. A., H. P. Linder & N. P. Barker. 1994. Haustorial synergids: An important character in the systematics of danthonioid grasses (Arundinoideae: Poaceae). Amer. J. Bot. 81: 1601–1610.

Vickery, J. W., S. W. L. Jacobs & J. Everett. 1986. Taxonomic studies in *Stipa* (Poaceae) in Australia. Telopea 3: 1–132.

Wang, Z. Y., F. Q. Zheng, J. P. Gao, X. Q. Wang, M. Wu, J. L. Zhang & M. M. Hong. 1994. Identification of two transposon-like elements in rice *Wx* gene. Sci. in China Ser. B-Chem. Life. Sci. & Earth Sci. 37: 437–447.

Watson, L. & M. J. Dallwitz. 1992. The Grass Genera of the World. CAB International, Wallingford, U.K.

——— & ———. 1999. Grass Genera of the World: Descriptions, Illustrations, Identification, and Information Retrieval; Including Synonyms, Morphology, Anatomy, Physiology, Phytochemistry, Cytology, Classification, Pathogens, World and Local Distribution, and References. http://biodiversityunoedu/delta/ Version: 18th August 1999.

———, H. T. Clifford & M. J. Dallwitz. 1985. The classification of the Poaceae: Subfamilies and supertribes. Austral. J. Bot. 33: 433–484.

Whistler, R. L., J. N. BeMiller & E. F. Paschall (editors). 1984. Starch: Chemistry and Technology. Academic Press, Orlando.

Wilson, W. A., S. E. Harrington, W. L. Woodman, M. Lee, M. E. Sorrells & S. R. McCouch. 1999. Inferences on the genome structure of progenitor maize through comparative analysis of rice, maize and the domesticated panicoids. Genetics 153: 453–473.

Zhang, W. 1996. Phylogeny and Classification of the Bamboos (Poaceae: Bambusoideae). Ph.D. Thesis, Iowa State University, Ames, Iowa.

———. 2000. Phylogeny of the grass family (Poaceae) from *rpl*16 intron sequence data. Molec. Phylogenet. Evol. 15: 135–146.

——— & L. G. Clark. 2000. Phylogeny and classification of the Bambusoideae (Poaceae). Pp. 35–42 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria.

Zuloaga, F. O., O. Morrone & L. Giussani. 2000. A cladistic analysis of the tribe Paniceae: A preliminary approach. Pp. 123–135 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses: Systematics and Evolution. CSIRO Publishing, Collingwood, Victoria.

Zurawski, G., M. T. Clegg & A. H. D. Brown. 1984. The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. Genetics 106: 735–749.

Appendix I. Taxa included. For each data set, species name, voucher, and reference are listed, as well as GenBank accession numbers for gene sequences. EAK = Elizabeth Kellogg; HPL = Peter Linder; JID = Jerrold Davis; LGC = Lynn Clark; NPB = Nigel Barker; PMP = Paul Peterson; RJS = Robert Soreng; SJ = Surrey Jacobs; WZ = Weiping Zhang; XL = Ximena Londoño; BBG = Berlin Botanic Garden; BHC = L. H. Bailey Hortorium Conservatory; FTG = Fairchild Tropical Garden; NTBG = National Tropical Botanical Garden (Hawaii); PI = USDA Plant Introduction Station (Pullman, Washington) as source of seed.

Genus	Species	Voucher	Reference	GenBank #
*ndh*F				
Flagellaria	*indica* L.	*LGC & WZ 1305* (ISC)	Clark et al. (1995)	U22007
Elegia	*stipularis* Mast.	*Eldenas 2* (BOL)	This paper	AF251443
Baloskion	*tetraphyllum* (Labill.) B. G. Briggs & L. A. S. Johnson	Kew-6565–1977 (BH)	This paper	AF251441
Joinvillea	*ascendens* Gaudich. ex Brongn. & Gris.	NTBG-800379 (living)	Clark et al. (1995)	U21973
Anomochloa	*marantoidea* Brongn.	*LGC 1299* (ISC)	Clark et al. (1995)	U21991
Streptochaeta	*angustifolia* Soderstr.	*LGC 1304* (ISC)	Clark et al. (1995)	U21982
Pharus	*latifolius* L.	*LGC 1302* (ISC)	Clark et al. (1995)	U21992
Guaduella	*marantifolia* Franch.	*Kobayashi et al. 1539* (ISC)	Clark et al. (2000)	AF164777
Puelia	*ciliata* Franch.	*Kobayashi et al. 1541* (ISC)	Clark et al. (2000)	AF164779
Eremitis	sp. nov.	*LGC & WZ 1343* (ISC)	Zhang & Clark (2000)	AF182353
Pariana	*radiciflora* Sagot ex Döll	*LGC & WZ 1344* (ISC)	Zhang & Clark (2000)	AF182354
Lithachne	*humilis* Soderstr.	*LGC 1298* (ISC)	Clark et al. (1995)	U21977
Olyra	*latifolia* L.	*XL & LGC 911* (ISC)	Clark et al. 1995	U21971
Buergersiochloa	*bambusoides* Pilg.	*Dransfield 1382* (K)	Zhang & Clark (2000)	AF182341
Arundinaria	*gigantea* (Walter) Muhl.	*WZ 8400703* (ISC)	Clark et al. (1995)	U21846
Chusquea	*latifolia* L. G. Clark	*LGC & XL 417* (ISC)	Clark et al. (1995)	U21989
Streptogyna	*americana* C. E. Hubb.	*Pohl & Davidse 12310* (ISC)	Clark et al. (1995)	U21965
Ehrharta	*calycina* Sm.	*NPB s.n.* (BOL)	Clark et al. (1995)	U21995
Oryza	*sativa* L.	Sugiura (1989)	Clark et al. (1995)	X15901
Leersia	*virginica* Willd.	*LGC 1316* (ISC)	Clark et al. (1995)	U21974
Phaenosperma	*globosum* Munro ex Benth.	*LGC 1292* (ISC)	Clark et al. (1995)	U22005
Brachyelytrum	*erectum* (Schreb.) P. Beauv.	*LGC 1330* (ISC)	Clark et al. (1995)	U22004
Lygeum	*spartum* L.	*RJS 3698* (BH)	This paper	AF251445
Nardus	*stricta* L.	BBG: *Royl & Schiers s.n. 1988* (B)	This paper	AF251446
Anisopogon	*avenaceus* R. Br.	*HPL 5590* (BOL)	This paper	AF251447
Ampelodesmos	*mauritanica* (Poir.) T. Durand & Schinz	BBG: *Royl & Schiers s.n.* 1988 (B)	This paper	AF251448
Stipa	*barbata* Desf.	PI-229468 (BH)	This paper	AF251449
Nassella	*viridula* (Trin.) Barkworth	PI-387938 (BH)	This paper	AF251450
Oryzopsis (=Piptatherum)	*racemosa* (Sm.) Ricker ex Hitchc.	*LGC & WZ 1288* (ISC)	Clark et al. (1995)	U21924
Brachypodium	*distachyon* (L.) P. Beauv.	PI-422452 (BH)	This paper	AF251451
Melica	*altisssima* L.	PI-325418 (BH)	This paper	AF251452
Glyceria	*striata* (Lam.) Hitchc.	*JID & RJS s.n.* (BH)	This paper	AF251453
Diarrhena	*obovata* (Gleason) Brandenburg	*LGC & WZ 1216* (ISC)	Clark et al. (1995)	U21998
Avena	*sativa* L.	material from R. Wise (ISU)	Clark et al. (1995)	U22000
Bromus	*inermis* Leyss.	PI-314071 (BH)	This paper	AF251454

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Hordeum	*vulgare* L.	material from R. Wise (ISU)	Clark et al. (1995)	U22003
Aristida	*purpurea* Nutt. var. *longiseta* (Steud.) Vasey ex Rothr.	*Gabel 2700* (ISC)	Clark et al. (1995)	U21966
Stipagrostis	*zeyheri* (Nees) DeWinter	*NPB 1133* (BOL)	This paper	AF251455
Amphipogon	*strictus* R. Br.	*HPL 5634* (BOL)	This paper	AF251456
Arundo	*donax* L.	*LGC s.n.* (ISC)	Clark et al. (1995)	U21997
Molinia	*caerulea* (L.) Moench	*LGC 1165* (ISC)	Clark et al. (1995)	U21994
Phragmites	*australis* (Cav.) Trin. ex Steud.	*LGC 1294* (ISC)	Clark et al. (1995)	U21996
Merxmuellera	*macowanii* (Stapf) Conert	*NPB 1008* (BOL)	This paper	AF251457
Karroochloa	*purpurea* (L.f.) Conert & Türpe	*HPL 5360* (BOL)	This paper	AF251458
Danthonia	*californica* Bolander	PI-232247 (BH)	This paper	AF251459
Austrodanthonia	*laevis* (Vickery) H. P. Linder	*HPL 5633* (BOL)	This paper	AF251460
Merxmuellera	*rangei* (Pilg.) Conert	*NPB 960* (GRA)	This paper	AF251461
Centropodia	*glauca* (Nees) Copt	*NPB 967* (BOL)	This paper	AF251462
Eragrostis	*curvula* (Schrad.) Nees	*LGC 1303* (ISC)	Clark et al. (1995)	U21988
Uniola	*paniculata* L.	*JID s.n.* (BH)	This paper	AF251463
Zoysia	*matrella* (L.) Merr.	*LGC 1174* (ISC)	Clark et al. (1995)	U21975
Distichlis	*spicata* (L.) E. Green subsp. *stricta* (Torr.) R. F. Thorne	*Allred s.n.* (BH)	This paper	AF251464
Pappophorum	*bicolor* E. Fourn.	*Pohl 12464* (ISC)	This paper	AF352581
Spartina	*pectinata* Link	*LaDuke s.n.* (BH)	This paper	AF251465
Sporobolus	*indicus* (L.) R. Br.	*LGC 1293* (ISC)	Clark et al. (1995)	U21983
Micraira	*lazaridis* L. G. Clark, Wendel & Craven	*LGC 1157* (ISC)	Clark et al. (1995)	U21972
Thysanolaena	*maxima* (Roxb.) Kuntze	FTG (living)	Clark et al. (1995)	U21984
Gynerium	*sagittatum* (Aubl.) P. Beauv.	*LGC & P. Asimbaya 1472* (ISC)	This paper	AF251466
Chasmanthium	*laxum* (L.) H. O. Yates	*D. Lewis s.n.* (ISC)	Clark et al. (1995)	U27296
Zeugites	*pittieri* Hack.	*LGC 1171* (ISC)	Clark et al. (1995)	U21987
Danthoniopsis	*petiolata* (J. B. Phipps) Clayton	*LGC 1173* (ISC)	Clark et al. (1995)	U22008
Panicum	*virgatum* L.	*LGC 1164* (ISC)	Clark et al. (1995)	U21986
Pennisetum	*alopecuroides* (L.) Spreng.	*RJS s.n.* (BH)	This paper	AF251467
Miscanthus	*japonicus* Andersson	Arnold Arboretum 301–80c (living)	Spangler et al. (1999)	AF117417
Zea	*mays* L. cv. 'B73'	Material from M. Lee (ISU)	Clark et al. (1995)	U21985
*rbc*L				
Flagellaria	*indica*	*Chase 206* (NCU)	Chase et al. (1993)	L12678
Elegia	*capensis* (Burm. f.) Schelpe	*Chase 209* (NCU)	Duvall & Morton (1996)	L12675
Baloskion	*tetraphyllum*	No voucher	Katiyama & Ogihara (1996)	D38296
Joinvillea	*plicata* (Hook. f.) Newell & B. C. Stone	*Thren 84* (NO)	Duvall & Morton (1996)	L01471
Anomochloa	*marantoidea*	*LGC 1299* (ISC)	Duvall & Morton (1996)	AF021875
Guaduella	*marantifolia*	*Kobayashi et al. 1539* (ISC)	Clark et al. (2000)	AF164778

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Puelia	*ciliata*	*Kobayashi et al. 1541* (ISC)	Clark et al. (2000)	AF164780
Lithachne	*humilis*	*LGC s.n.* (ISC)	Duvall & Morton (1996)	U13231
Bambusa	*multiplex* (Lour.) Raeusch. ex Schult. & Schult. f.	*Sanders 62–616* (UCR)	Duvall & Morton (1996)	M91626
Chusquea	*circinata* Soderstr. & C. Calderón	Quail Botanic Garden (living)	Duvall & Morton (1996)	U13227
Oryza	*sativa*	No voucher	Nishizawa & Hirai (1987)	D00207
Leersia	*oryzoides* (L.) Sw.	*LGC s.n.* (ISC)	Duvall & Morton (1996)	U13228
Stipa	*dregeana* Steud. var. *dregeana*	*McDowell s.n.* (BOL)	Barker et al. (1995)	
Avena	*sativa*	No voucher	Duvall et al. (1993)	L15300
Bromus	*inermis* Leyss.	No voucher	Seberg & Linde-Laursen (1996)	Z49836
Hordeum	*vulgare*	No voucher	Zurawski et al. (1984)	X00630
Aristida	*congesta* Roem. & Schult.	*NPB 1130* (BOL)	Barker et al. (1995)	U31359
Stipagrostis	*zeyheri*	*NPB 1133* (BOL)	Barker et al. (1995)	U31378
Amphipogon	*strictus*	*HPL 5634* (BOL)	Barker (1997)	U88403
Arundo	*donax*	*NPB 1131* (BOL)	Barker et al. (1995)	U31360
Moliniopsis	*japonica* (Hack.) Hayata	*Kobayashi 1253*	Barker et al. (1995)	U31439
Phragmites	*australis*	*NPB 1132* (BOL)	Barker et al. (1995)	U29900
Merxmuellera	*macowanii*	*NPB 1008* (BOL)	Barker et al. (1995)	U31438
Karroochloa	*purpurea*	*HPL 5360* (BOL)	Barker et al. (1995)	U31437
Danthonia	*spicata* (L.) P. Beauv. ex Roem. & Schult.	*EAK V10* (GH)	Barker et al. (1995)	U31102
Centropodia	*glauca*	*HPL 5410* (BOL)	Barker et al. (1995)	U31100
Eragrostis	*capensis* (Thunb.) Trin.	*NPB 1135* (BOL)	Barker et al. (1995)	U31104
Enneapogon	*scaber* Lehm.	*NPB 1023* (BOL)	Barker et al. (1995)	U31103
Eriachne	*triodioides* Domin	*EAK s.n.* (GH)	This paper	AF352580
Thysanolaena	*maxima*	Kew 1979–3225 Warr (living)	Barker et al. (1995)	U31380
Gynerium	*sagittatum*	Kew 1991–1276 Kall (living)	Barker et al. (1995)	U31105
Chasmanthium	*latifolium* (Michx.) H. O. Yates	*Snow 5944*	Barker et al. (1995)	U31101
Pennisetum	*glaucum* (L.) R. Br.	No voucher	Doebley et al. (1990)	L14623
Sorghum	*bicolor* (L.) Moench	No voucher	Lou et al. (1989)	1515164A
Zea	*mays*	No voucher	Gaut et al. (1992)	Z11973
rpoC2				
Joinvillea	*plicata*	No voucher	Barker et al. (1999)	AF001864
Olyra	*latifolia*	*HPL 5742* (BOL)	Barker et al. (1999)	U90825
Bambusa	*vulgaris* Schrad. ex J. C. Wendl.	Durban Botanic Garden (living)	Barker et al. (1999)	U90824
Ehrharta	*dura* Nees ex Trin.	*NPB 1118* (BOL)	Barker et al. (1999)	AF064761
Oryza	*sativa*	No voucher	Hiratsuka et al. (1989)	X15901
Lygeum	*spartum*	Kew (living)	Cummings et al. (1994)	L25381
Nardus	*stricta*	Kew (living)	Cummings et al. (1994)	L25382
Anisopogon	*avenaceus*	*HPL 5590* (BOL)	Barker et al. (1999)	U92263
Stipa	*dregeana*	*McDowell s.n.* (BOL)	Barker et al. (1999)	U90826
Briza	*maxima* L.	*EAK s.n.* (GH)	Cummings et al. (1994)	L25376
Bromus	*tectorum* L.	*EAK s.n.* (GH)	Cummings et al. (1994)	L25377

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Aristida	*congesta* Roem. & Schult. subsp. *barbicollis* (Trin. & Rupr.) DeWinter	*NPB 1130* (BOL)	Barker et al. (1999)	U90827
Stipagrostis	*zeyheri* subsp. *zeyheri*	*NPB 1133* (BOL)	Barker et al. (1999)	U90828
Amphipogon	*strictus*	*HPL 5634* (BOL)	Barker et al. (1999)	U94335
Arundo	*donax*	*NPB 1131* (BOL)	Barker et al. (1999)	U92264
Moliniopsis	*japonica*	*Kobayashi 1253*	Barker et al. (1999)	U95081
Phragmites	*australis*	*NPB 1132* (BOL)	Barker et al. (1999)	U95130
Merxmuellera	*macowanii*	*NPB 1008* (BOL)	Barker et al. (1999)	U95076
Karroochloa	*purpurea*	*HPL 5360* (BOL)	Barker et al. (1999)	U94824
Danthonia	*spicata*	*EAK V10* (GH)	Barker et al. (1999)	U93362
Austrodanthonia	*laevis*	*HPL 5633* (BOL)	Barker et al. (1999)	U96313
Merxmuellera	*rangei*	*NPB 960* (GRA)	Barker et al. (1999)	U95077
Centropodia	*glauca*	*HPL 5410* (BOL)	Barker et al. (1999)	U92265
Eragrostis	*capensis*	*NPB 1135* (BOL)	Barker et al. (1999)	U96317
Enneapogon	*scaber*	*NPB 1023* (BOL)	Barker et al. (1999)	U96319
Spartina	*alterniflora*	*EAK s.n.* (GH)	Cummings et al. (1994)	L25386
Micraira	*lazaridis*	*LGC 1157* (ISC)	Barker et al. (1999)	U96318
Thysanolaena	*maxima*	Kew, 1979–3225 Warr (living)	Barker et al. (1999)	U96315
Gynerium	*sagittatum*	Kew, 1991–1276 Kall (living)	Barker et al. (1999)	U94392
Chasmanthium	*latifolium*	*Snow 5944*	Barker et al. (1999)	U94334
Panicum	*maximum* Jacq.	*NPB 1125* (BOL)	Barker et al. (1999)	AF000021
Pennisetum	sp.	No voucher	Cummings et al. (1994)	L25383
Sorghum	*bicolor*	No voucher	Chen et al. (1993)	Z14983
Zea	*mays*	No voucher	Igloi et al. (1990)	X86563
Phytochrome B				
Flagellaria	*indica*	*RJS 77 394* (BH)	Mathews & Sharrock (1996)	U61203
Joinvillea	*ascendens*	*Moore 10438* (NY)	Mathews & Sharrock (1996)	U61205
Anomochloa	*marantoidea*	*LGC 1299* (ISC)	Mathews et al. (2000)	AF137291
Streptochaeta	*angustifolia*	*LGC 1304* (ISC)	Mathews et al. (2000)	AF137328
Pharus	*lappulaceus* Aubl.	*LGC 1329* (ISC)	Mathews et al. (2000)	AF137321
Puelia	*ciliata*	*Kobayashi et al. 1541* (ISC)	Mathews et al. (2000)	AF137324
Eremitis	sp. nov.	*LGC & WZ 1343* (ISC)	Mathews et al. (2000)	AF137304
Pariana	*radiciflora*	*LGC & WZ 1344* (ISC)	Mathews et al. (2000)	AF137317
Lithachne	*pauciflora* (Sw.) P. Beauv.	*LGC 1297* (ISC)	Mathews et al. (2000)	AF137307
Olyra	*latifolia*	*XL & LGC 911* (ISC)	Mathews et al. (2000)	AF137315
Buergersiochloa	*bambusoides*	*Dransfield 1382* (K)	Mathews et al. (2000)	AF137295
Pseudosasa	*japonica* (Sieb. & Zucc. ex Steud.) Makino ex Nakai	*EAK V6* (A)	Mathews et al. (2000)	AF137323
Chusquea	*oxylepis* (Hack.) Ekman	*LGC 1069* (ISC)	Mathews et al. (2000)	AF137298
Streptogyna	*americana*	*Johnston 433*	Mathews et al. (2000)	AF137329
Ehrharta	*erecta* Lam.	*EAK V44* (GH)	Mathews et al. (2000)	AF137302
Oryza	*sativa*	no voucher	Dehesh et al. (1991)	X57563
Lygeum	*spartum*	*RJS 3698* (BH)	Mathews et al. (2000)	AF137309
Nardus	*stricta*	BBG: *Royl & Schiers s.n.*	Mathews et al. (2000)	AF137313
Anisopogon	*avenaceus*	*HPL 5590* (BOL)	Mathews et al. (2000)	AF137290
Nassella	*viridula*	*Lavin s.n.* (MONT)	Mathews et al. (2000)	AF137314
Brachypodium	*pinnatum* (L.) P. Beauv.	*PI-440176* (GH)	Mathews et al. (2000)	AF137294
Melica	*cupanii* Guss.	*PI-383702* (BH)	Mathews et al. (2000)	AF137310

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Glyceria	*grandis* S. Watson	*JID & RJS s.n.* (BH)	Mathews et al. (2000)	AF137305
Diarrhena	*obovata*	*LGC & WZ 1216* (ISC)	Mathews et al. (2000)	AF137301
Phalaris	*arundinacea* L.	*RJS 3427* (BH)	Mathews et al. (2000)	AF137320
Bromus	*inermis*	*Lavin s.n.* (MONT)	Mathews et al. (2000)	U61193
Triticum	*aestivum* L.	*Mason-Gamer s.n.* (GH)	Mathews et al. (2000)	AF137331
Aristida	*purpurea* subsp. *longiseta*	*Lavin s.n.* (MONT)	Mathews et al. (2000)	AF137292
Molinia	*caerulea*	*RJS 3305* (BH)	Mathews et al. (2000)	AF137312
Phragmites	*australis*	*Keller s.n.* (GH)	Mathews et al. (2000)	AF137322
Danthonia	*spicata*	*EAK V10* (GH)	Mathews et al. (2000)	AF137299
Eragrostis	*cilianensis* (All.) Vignolo ex Janch.	*Lavin s.n.* (MONT)	Mathews et al. (2000)	U61200
Sporobolus	*giganteus* Nash	*PMP 10008* (US)	Mathews et al. (2000)	AF137327
Thysanolaena	*maxima*	*Farnsworth s.n.* (GH)	Mathews et al. (2000)	AF137330
Chasmanthium	*latifolium*	*EAK V13* (A)	Mathews et al. (2000)	AF137297
Danthoniopsis	*dinteri* (Pilg.) C. E. Hubb.	*PI-207548* (GH)	Mathews et al. (2000)	AF137300
Panicum	*capillare* L.	*Lavin s.n.* (MONT)	Mathews et al. (2000)	AF137316
Pennisetum	*alopecuroides*	*EAK s.n.* (A)	Mathews et al. (2000)	AF137318
Miscanthus	*japonicus*	Arnold Arboretum 301–80C (living)	Mathews et al. (2000)	AF137311
Zea	*mays*	*Lavin s.n.* (MONT)	Mathews et al. (2000)	AF137332
Chloroplast restriction site polymorphisms				
Flagellaria	*indica*	BHC-77394	Soreng & Davis (1998)	
Baloskion	*tetraphyllum*	Kew-6565–1977 (BH)	Soreng & Davis (1998)	
Joinvillea	*ascendens*	NTBG-800379 (*H. Moore 10438*)	Davis & Soreng (1993)	
Anomochloa	*marantoidea*	*LGC 1299* (ISC)	Soreng & Davis (1998)	
Streptochaeta	*sodiroana* Hack.	*PMP 9525* (US)	Soreng & Davis (1998)	
Pharus	*latifolius*	BHC from USZ	Davis & Soreng (1993)	
Eremitis	sp.	USNHG-153, *Soderstrom 2182* (US) or USNHG-286 (US)	Soreng & Davis (1998)	
Lithachne	*humilis*	BHC from U. S. National Zoological Gardens	Davis & Soreng (1993)	
Olyra	*latifolia*	*PMP 7311* (US)	Soreng & Davis (1998)	
Pseudosasa	*japonica*	BHC-71467	Davis & Soreng (1993)	
Chusquea	aff. *subulata* L. G. Clark	*PMP 9499* (US)	Soreng & Davis (1998)	
Ehrharta	*calycina*	*PI-208983* (BH)	Soreng & Davis (1998)	
Oryza	*sativa*	no voucher	Hiratsuka et al. (1989)	
Leersia	*virginica*	*RJS 3399* (BH)	Davis & Soreng (1993)	
Brachyelytrum	*erectum*	*RJS 3427* (BH)	Davis & Soreng (1993)	
Lygeum	*spartum*	*RJS 3698* (BH)	Soreng & Davis (1998)	
Nardus	*stricta*	BBG: seed from *Royl & Schiers s.n.* 1988, *Hempel s.n.* 1987 (B)	Davis & Soreng (1993)	
Anisopogon	*avenaceus*	*HPL 5590* (BOL)	Soreng & Davis (1998)	
Ampelodesmos	*mauritanica*	BBG: *Royl & Schiers s.n.* 1988 (B)	Soreng & Davis (1998)	
Stipa	*barbata*	*PI-229468* (BH)	Davis & Soreng (1993)	
Nassella	*viridula*	*PI-387938* (BH)	Soreng & Davis (1998)	
Piptatherum	*miliaceum* (L.) Coss.	*PI-284145* (BH)	Davis & Soreng (1993)	
Brachypodium	*pinnatum*	*PI-440170* (BH)	Davis & Soreng (1993)	
Melica	*altissima*	*PI-325418* (BH)	Davis & Soreng (1993)	
Glyceria	*striata*	*JID & RJS s.n.* (BH)	Davis & Soreng (1993)	
Diarrhena	*obovata*	Seed from *Tiedye 5186* (DAO)	Davis & Soreng (1993)	

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Avena	*barbata* Pott ex Link	No voucher	Soreng & Davis (1998)	
Bromus	*inermis*	*RJS 3428* (BH), PI-314071 (BH)	Davis & Soreng (1993)	
Triticum	*aestivum* L. cv. 'Susquehanna'	*RJS s.n.* (BH)	Soreng & Davis (1998)	
Aristida	*purpurea*	*Allred s.n.* (BH)	Soreng & Davis (1998)	
Amphipogon	*strictus*	*HPL 5634* (BOL)	Soreng & Davis (1998)	
Arundo	*donax*	FTG-83–130 (BH)	Soreng & Davis (1998)	
Molinia	*caerulea*	*RJS 3305* (BH)	Soreng & Davis (1998)	
Phragmites	*australis*	*RJS 3884* (BH)	Davis & Soreng (1993)	
Danthonia	*californica*	*PI-232247* (BH)	Davis & Soreng (1993)	
Eragrostis	*curvula*	*PI-365034* (BH)	Davis & Soreng (1993)	
Uniola	*paniculata*	*JID s.n.* (BH)	Soreng & Davis (1998)	
Zoysia	sp.	*JID s.n.* (BH)	Soreng & Davis (1998)	
Distichlis	*spicata* subsp. *stricta*	*Allred s.n.* (BH)	Soreng & Davis (1998)	
Spartina	*pectinata*	*LaDuke s.n.* (BH)	Soreng & Davis (1998)	
Sporobolus	*giganteus*	*PMP 10008* (US)	Soreng & Davis (1998)	
Chasmanthium	*latifolium*	Cornell University gardens (living)	Davis & Soreng (1993)	
Panicum	*virgatum*	USDA 421520 (BH)	Soreng & Davis (1998)	
Pennisetum	*alopecuroides*	*RJS s.n.* (BH)	Davis & Soreng (1993)	
Miscanthus	*sinensis* Andersson var. *gracillimus* Hitchc.	*RJS s.n.* (BH)	Davis & Soreng (1993)	
ITS				
Joinvillea	*plicata*	*Wilson 7126*	Hsiao et al. (1999)	AF019784
Streptochaeta	*sodiroana*	*PMP & Annable 9525* (US)	Hsiao et al. (1999)	AF019785
Pharus	*latifolius*	*PMP & Annable 6944* (US)	Hsiao et al. (1999)	AF019786
Lithachne	*humilis*	Utah State University s.n. (living)	Hsiao et al. (1999)	AF019787
Chusquea	*latifolia*	*LGC & XL 417* (ISC)	Hsiao et al. (1999)	AF019788
Microlaena	*stipoides* (Labill.) R. Br.	Kew 1973–15875 (living)	Hsiao et al. (1999)	AF019791
Oryza	*sativa*	No voucher	Takaiwa et al. (1985)	
Leersia	*hexandra* Sw.	*Jacobs 7782*	Hsiao et al. (1999)	AF019793
Brachyelytrum	*erectum*	Intermountain Herbarium 1669	Hsiao et al. (1999)	AF019794
Lygeum	*spartum*	*Catalan 1593*	Hsiao et al. (1999)	AF019797
Nardus	*stricta*	Intermountain Herbarium 203443	Hsiao et al. (1999)	AF019796
Anisopogon	*avenaceus*	*Dalby 94/01*	Hsiao et al. (1999)	AF019800
Ampelodesmos	*mauritanica*	Kew 150–90.00982 (living)	Hsiao et al. (1999)	AF019799
Stipa	*ichu* (Ruiz & Pavón) Kunth	*Renvoize & Flores 5301* (K)	Hsiao et al. (1999)	AF019803
Nassella	*leucotricha* (Trin. & Rupr.) R. W. Pohl	*Houck s.n.*	Hsiao et al. (1999)	L36520
Piptatherum	*songaricum* (Trin. & Rupr.) Roshev. ex Nikitina	*Hsiao 199*	Hsiao et al. (1999)	AF019802
Brachypodium	*mexicanum* (Roem. & Schult.) Link	University of Leicester Botanic Gardens 347	Hsiao et al. (1999)	AF019805
Melica	*californica* Scribn.	*Curto 719*	Hsiao et al. (1999)	L36518
Glyceria	*striata*	*Curto 826*	Hsiao et al. (1999)	L36516
Diarrhena	*americana* P. Beauv.	Intermountain Herbarium 218465	Hsiao et al. (1999)	AF019798

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Avena	*longiglumis* Durieu	*Fritz*, CN	Hsiao et al. (1999)	Z11758
Bromus	*inermis*	*Hsiao 103*	Hsiao et al., (1994)	L11579
Hordeum	*vulgare*	*Hsiao 200*	Chatterton et al. (1992)	Z11759
Aristida	*purpurea*	Intermountain Herbarium 209381	Hsiao et al., 1999	AF019807
Stipagrostis	*zeyheri* subsp. *zeyheri*	*NPB 1133*	Hsiao et al. (1999)	AF019845
Amphipogon	*caricinus* F. Muell.	*Macfarlane 2155*	Hsiao et al. (1998)	AF019849
Arundo	*donax*	*Hsiao 196, Evans s.n.*	Hsiao et al. (1999)	AF019809
Molinia	*caerulea*	Kew1973–10386	Hsiao et al. (1999)	AF109857
Phragmites	*australis*	*Chatterton s.n.*	Hsiao et al. (1999)	AF019810
Merxmuellera	*macowanii*	Kew 142–83.01715	Hsiao et al. (1998)	AF019863
Karroochloa	*purpurea*	*HPL 5360*	Hsiao et al. (1998)	AF019874
Danthonia	*californica*	*Curto 974*	Hsiao et al. (1999)	AF019813
Rytidosperma	*pumilum* (Kirk) H. P. Linder	*HPL 5747*	Hsiao et al. (1998)	AF019878
Merxmuellera	*rangei*	*NPB 960* (GRA)	Hsiao et al. (1998)	AF019862
Centropodia	*glauca*	*NPB 967*	Hsiao et al. (1998)	AF019861
Eragrostis	*dielsii* Pilg. ex Diels & Pritz.	*Jacobs 7195*	Hsiao et al. (1999)	AF019834
Spartina	*gracilis* Trin.	Intermountain Herbarium 194828	Hsiao et al. (1999)	AF019844
Sporobolus	*airoides* (Torr.) Torr.	*Curto s.n.*	Hsiao et al. (1999)	AF019842
Eriachne	*triseta* Nees ex Steud.	*Jacobs 7184*	Hsiao et al. (1999)	AF019818
Micraira	*subulifolia* F. Muell.	*Clarkson 10300*	Hsiao et al. (1999)	AF019859
Thysanolaena	*maxima*	Kew1979–3225	Hsiao et al. (1999)	AF019854
Gynerium	*sagittatum*	Kew1991–1276Kall	Hsiao et al. (1999)	AF019853
Chasmanthium	*latifolium*	Intermountain Herbarium 216008	Hsiao et al. (1999)	AF019815
Panicum	*bisulcatum* Thunb.	*Hsiao 160*, PI-19486	Hsiao et al. (1999)	AF019829
Pennisetum	*setaceum* (Forssk.) Chiov.	*Curto 976*	Hsiao et al. (1999)	AF019833
Miscanthus	*sinensis*	*Evans s.n.*	Hsiao et al. (1999)	AF019822
Zea	*mays* L. subsp. *mexicana* (Schrad.) Iltis	*Hsiao 197*	Hsiao et al. (1999)	AF019817
GBSSI				
Anomochloa	*marantoidea*	*LGC 1299* (ISC)	Mason-Gamer et al. (1998)	AF079290
Pharus	*lappulaceus*	*LGC 1329* (ISC)	Mason-Gamer et al. (1998)	AF079298
Eremitis	sp. nov.	*LGC & WZ 1343* (ISC)	Mason-Gamer et al. (1998)	AF079295
Pariana	*radiciflora*	*LGC & WZ 1344* (ISC)	Mason-Gamer et al. (1998)	AF079297
Chusquea	*exasperata* L. G. Clark	*LGC et al. 1093* (ISC)	Mason-Gamer et al. (1998)	AF079293
Oryza	*sativa*	No voucher	Wang et al. (1994)	X65183
Lygeum	*spartum*	*RJS 3698*	Mason-Gamer et al. (1998)	AF079289
Melica	*cupanii*	PI-383702 (A)	Mason-Gamer et al. (1998)	AF079296
Glyceria	*grandis*	*JID & RJS s.n.*	Mason-Gamer et al. (1998)	AF079291
Hordeum	*vulgare*	No voucher	Rohde et al. (1988)	X07932
Merxmuellera	*macowanii*	*NPB 1008* (BOL)	This paper	AF353520
Karroochloa	*purpurea*	*HPL 5360* (BOL)	This paper	AF353519
Austrodanthonia	*laevis*	*HPL 5633* (BOL)	This paper	AF353517

Appendix I. Continued.

Genus	Species	Voucher	Reference	GenBank #
Merxmuellera	*rangei*	*NPB 960* (GRA)	This paper	AF353521
Centropodia	*glauca*	*NPB 967* (BOL)	This paper	AF353518
Danthoniopsis	*dinteri*	*PI-207548* (A)	Mason-Gamer et al. (1998)	AF079251
Pennisetum	*alopecuroides*	Park Seed 3650 (A)	Mason-Gamer et al. (1998)	AF079288
Sorghum	*bicolor*	PI-156519 (A)	Mason-Gamer et al. (1998)	AF079258
Zea	*mays*	No voucher	Klösgen et al. (1986)	X03935

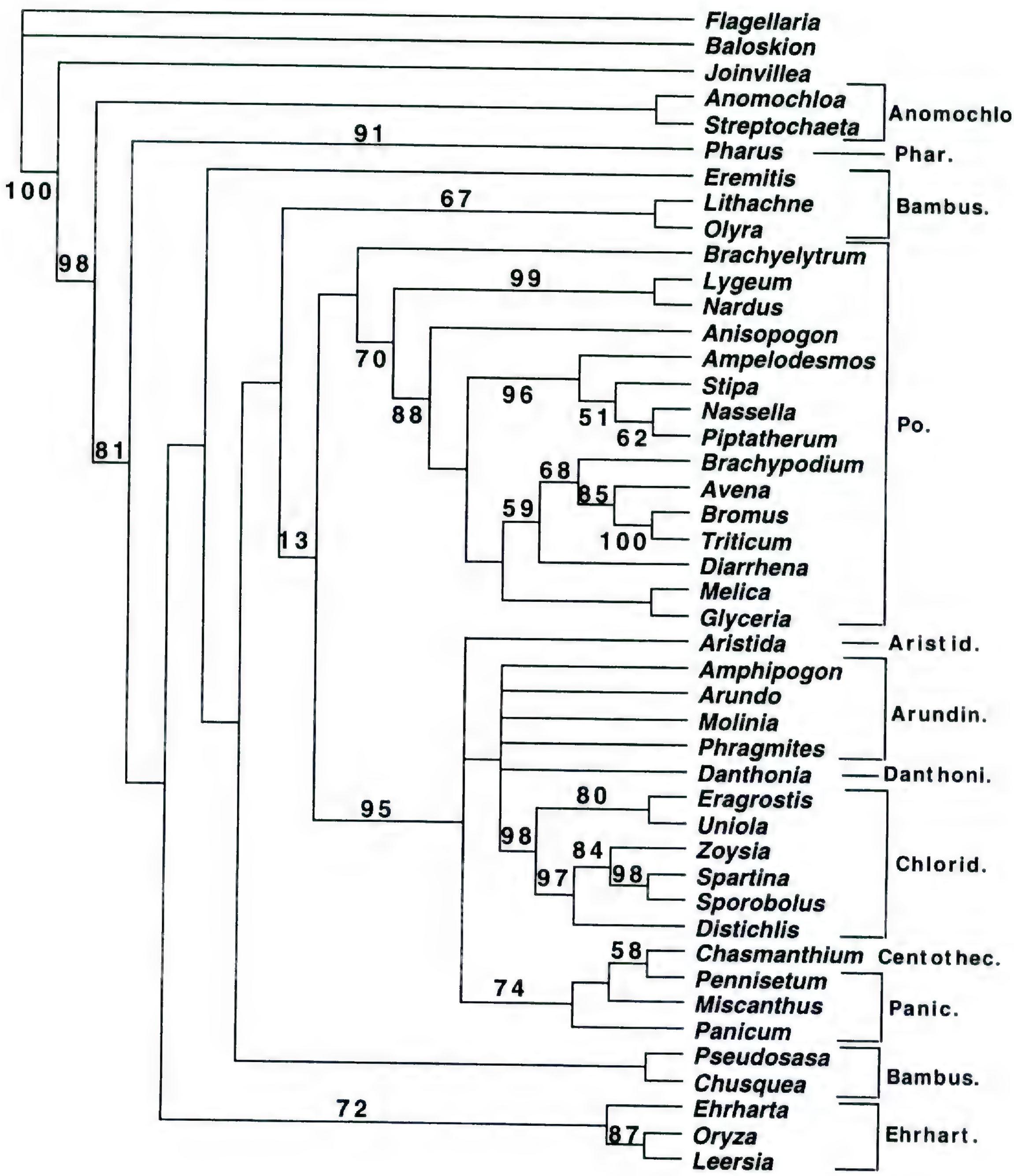
Appendix II. Matrix of structural characters, as assembled for analysis in NONA (Goloboff, 1993). Taxa in the matrix appear in groupings according to what was known about the phylogeny at the time the taxon sampling list was prepared. Thus, the four outgroups come first, followed by the early-diverging taxa, then bambusoids, rices, pooids, etc. Abbreviations of taxon names and associated underlines are required by the program. Characters and character states are described in Table 4, and are optimized on the cladogram in Figure 3. Codes used for polymorphisms (presence of two or more states) and subset ambiguities (when one or more states are not present, but the observed attribute cannot be assigned to any of the states not eliminated) are as follows: ? = unobserved; - = inapplicable; \ = intermediate/uncertain homology/unassignable to defined states; A = [01]; C = [03]; D = [12]; E = [13]; F = [23]; H = [034]; J = [234]; K = [0134]; L = [14]; N = [29]; Q = [07]; R = [012]; S = [57]; T = [127].

Character numbers	000000000111111111222222222233333333334444444444555
Taxon	123456789012345678901234567890123456789012345678901234
Flagellaria	\01-01A0---------0\\\\1111003130-0----??00?-0050-\000
Elegia	000--0-0---------0\\\\1100103310-0----??1-0---?0-???1
Baloskion	0?0--0-0----- --0\\\\1100102D2?-0- 011-0---T0-?0?0
Joinvillea	01000110---------0\\\\11110A3130-0----???110\140-1100
Anomochloa	0001011\-\\\0---\0\\\\10110\111?011100?101101140-0110
Streptochaeta	01010110-\\\\---\0\\\\1111013112?010101?10?100110-0110
Pharus	0000011111010--A0130??111101311?01110101110-1120-0110
Guaduella	0100011101100--10130111111002212?01???????110A1?0-???1
Puelia	0100111101110--1013011111101F212?01???????10-1120-0??1
Eremitis	0100011101010--001301100010\111?011101?1011011\0-01?1
Pariana	010001110A010 0\130111111A211?011101??011011D0-0??1
Lithachne	01000111A1010--1113011001101 2F1?0111010?01101110-0111
Olyra	0100011101010--1113011001101 2F1?011101010110 1110-01?1
Buergersiochloa	0100011101011101013011 00A10122?1?01???????11011?0-0??1
Pseudosasa	1100111110100A10101301111111013210011101?301101120 01?1
Chusquea	10001111011 1A--1013011001101221?0111010?01101100-01?1
Streptogyna	010011A10100111101301100010 1F11?011101?K11100120-0??1
Ehrharta	0A000101011111010120111111002210010101011A10A020-01?1
Oryza	010001A101\1A10\1120111111 0A221?011001010110 1020-0110
Leersia	0100010101\1A10\0120A1000100231?011001010110A020-?1?0
Phaenosperma	0A00011101010--001301\0011002D1?01110104?10-0020-???1
Brachyelytrum	0000010101011101012011001100221?011A01?4110-0010-?111
Lygeum	000001010\000--1?0----00110\121?011000011111 0000-0111
Nardus	010001010001110 1?0----00110\111?011000011110 0030-01??
Anisopogon	0100010101011311013010001100F21001?????1?10-00-0-0111
Ampelodesmos	000001010100 11A10130100011002 21?0110000?110-0020-?1?1
Stipa	010001010101111A101F0100011 00J2100110000K110-00R0 ?1?1
Nassella	010001010101111A10120100011 00221?0110000C110-00\0-01?1
Piptatherum	010001010101A10101301000010 0221?01???01110-0020-?1?1
Brachypodium_d	01000101010011 01?1201?00110221?01A00000110-00S0-01?1
Melica_a	01100101010A0--0012100001100231?011000011A0-0090-01?1

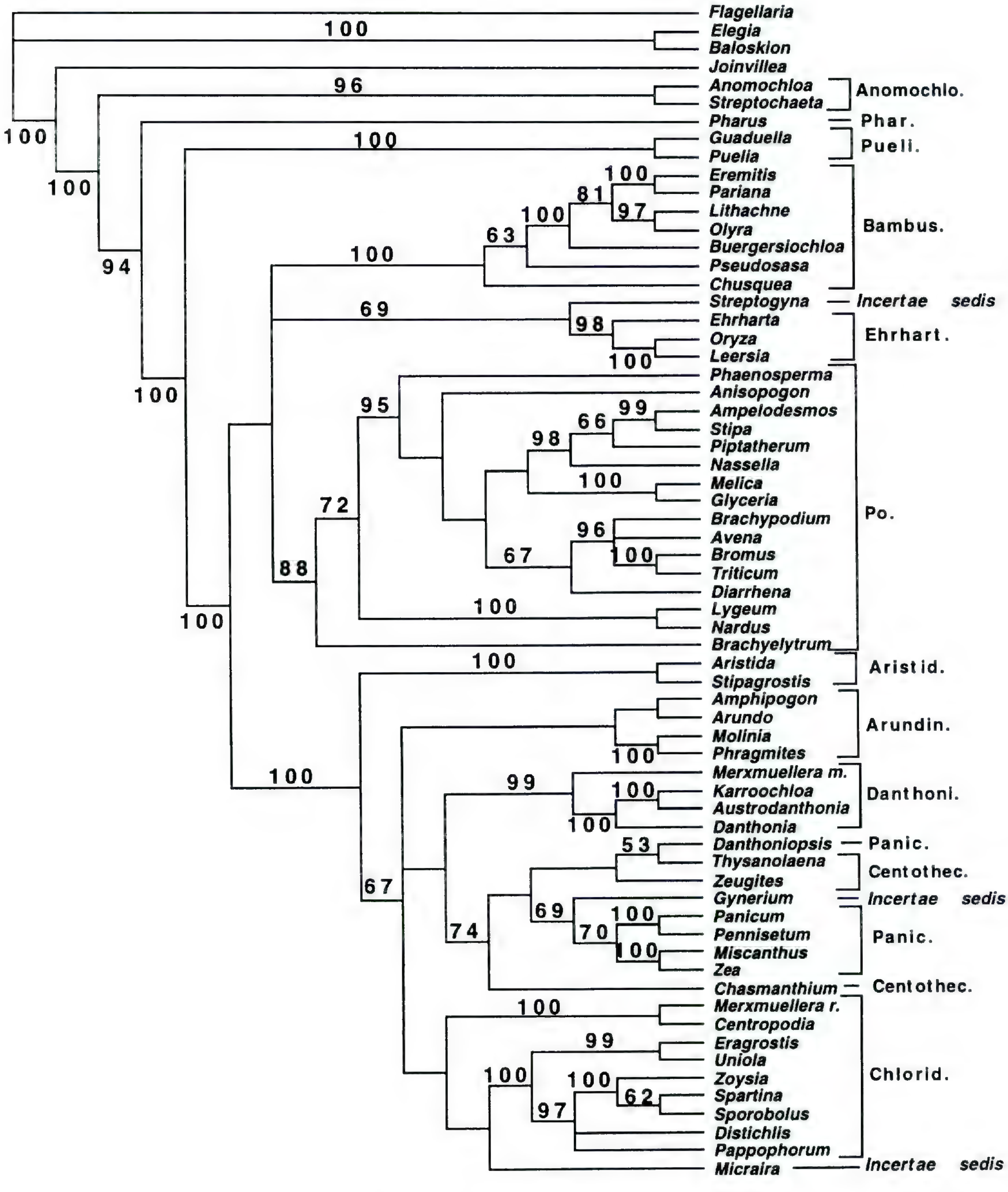
Appendix II. Continued.

Character numbers	000000000111111111122222222223333333333444444444455555
Taxon	123456789012345678901234567890123456789012345678901234
Glyceria_s	0110010101000--1012A000001002310?011000011A0-0000-01?1
Diarrhena	01000101010A0--101201000A100221?011A0A01110-0000-01?1
Avena	0100010101001E2101201000110221?011A0\111A0-0070-01?1
Bromus	011001010100A1R101201000110221?010000001A0-0070-01?1
Triticum	010001010000A1010120100011022l?01100\001A0-0070-01?1
Aristida	000A010101011E0111201100110022100101100l1A100012-11?1
Stipagrostis	0A010101010113011A2011001100221?010110?D11100013O???1
Amphipogon	0101010101013A1112000001101221?110110?1?11100-0-?1?1
Arundo	110001010100AEA1012001001100221?110110?1?A100020-?111
Molinia	010101010100A101?12A010011002210101100111100090-1111
Phragmites	\101010101l0A1010120010011012210\10110011110l020-1111
M_merxmuellera_m_	0001010101001E1101201100110022110l??????1100?0-???1
Karroochloa	0101010101001E1101200100110022111101l0???1100060-???1
Danthonia	0101010101001311012001001100221101011OOD11100040-11?1
Austrodanthonia	0101010101001311012001001100221111011O???1100020-???1
R_merxmuellera_r_	0?01010101001E1101200100110022l??????????10-0060-???1
Centropodia	0001010101001111012001001100221011????L?1100063O???1
Eragrostis	0\01010101000--1012001001100221?1111100111lA000301111
Uniola	0\01010101100--1012001001101221?110110??11110003\?1?1
Zoysia	000A010A0001110000----00A10A221?1111100D11110003l?1?1
Distichlis	0000010101000--1012001001100221?1111100H?11100030?1?1
Pappophorum	0?010101010A120101200\001100221?\111100??111000E-???1
Spartina	010101010001O--000----00110122l?1111100111A100Q31?1?1
Sporobolus	000A010101010--10A200100AA002D1?1111100E11110093A11?1
Eriachne	0A01A101010AA1A101200100A100221?010111??11100O?5-????
Micraira	000A01A101AA0--100----000101221?0101???H11100000-???1
Thysanolaena	1100111101010--101200000A100221?11???????11010D0-1111
Gynerium	100AA1\101000--101201\00010022l?11???????11010\0-???1
Chasmanthium_l	0100010101100--1012001001001221?111111031110002O-1111
Zeugites	010001110101A10A01200\00110122l?111101?1?11000\0-???1
Danthoniopsis	0A01A1A1A1111E1111200100110022l?01?????4111000NL-1??1
Panicum	0100010101110--01120A1001100221011011l0E111000\3011?1
Pennisetum	010A01010111A1001A200100110A221?1101110311100091-11?1
Miscanthus	0000010111111110012001001100221?110111?211100051-11?1
Zea	010001011A110--0?120010011012l1?\101110311100001-11?1

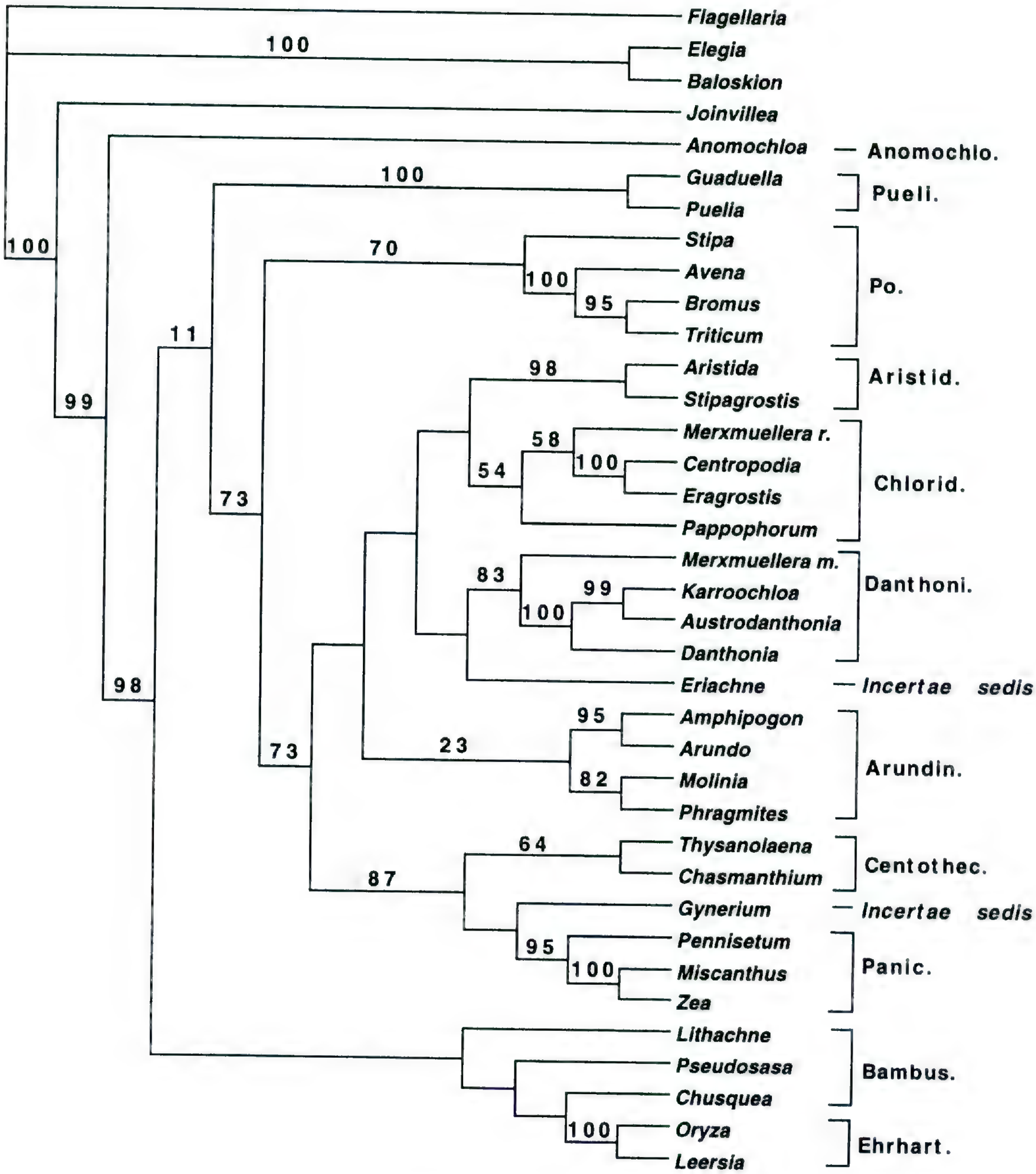
Appendix III. Consensus trees for individual data sets and combinations of data sets. Numbers above branches indicate percent of 500 bootstrap replicates, except for K (all molecular data), for which 1000 replicates were done. Tree statistics are listed in Table 3. The GPWG classification is overlain on each tree for comparison with Figure 2. —A. Chloroplast restriction sites; strict consensus of seven trees. —B. *ndh*F; strict consensus of 16 trees. —C. *rbc*L; single most parsimonious tree. —D. *rpo*C2; strict consensus of 33 trees. —E. Phytochrome B; single most parsimonious tree. —F. ITS; strict consensus of 24 trees. —G. GBSSI, single most parsimonious tree. —H. Structural data; strict consensus of 38,000 trees. —I. Chloroplast data; strict consensus of two trees. —J. Nuclear data; strict consensus of eight trees. —K. All molecular data; strict consensus of six trees.



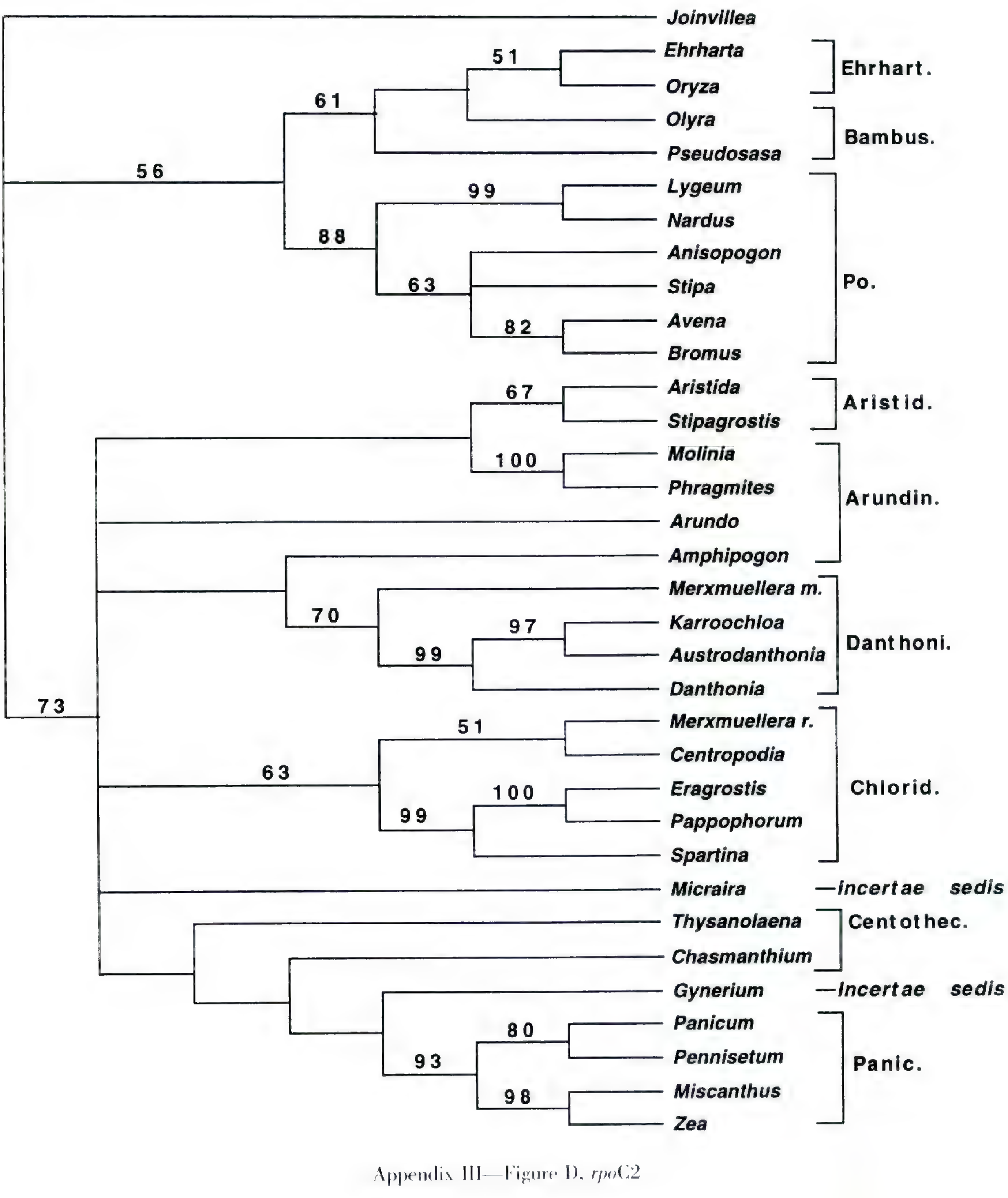
Appendix III—Figure A, cp restriction sites



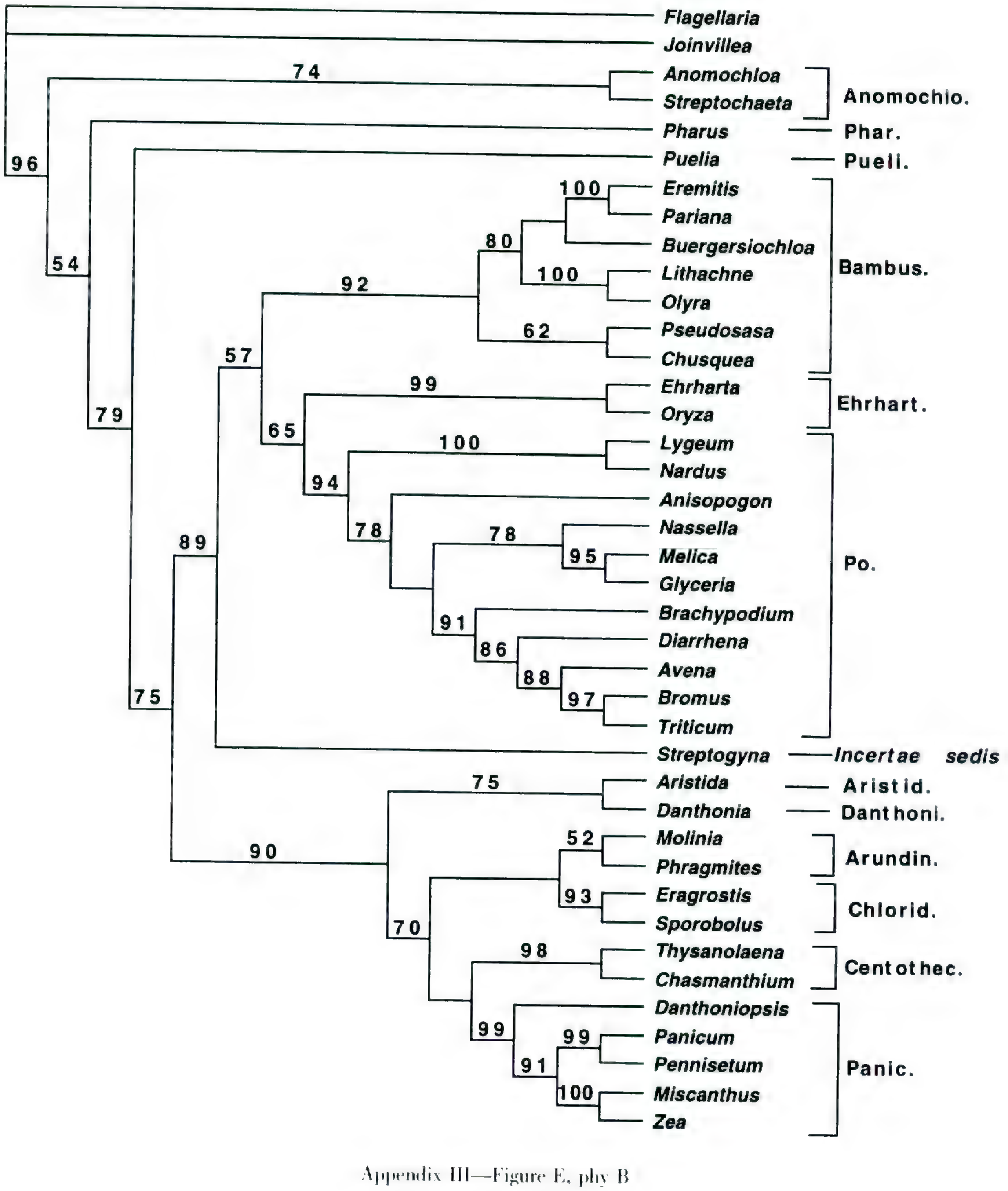
Appendix III—Figure B, *ndh*F



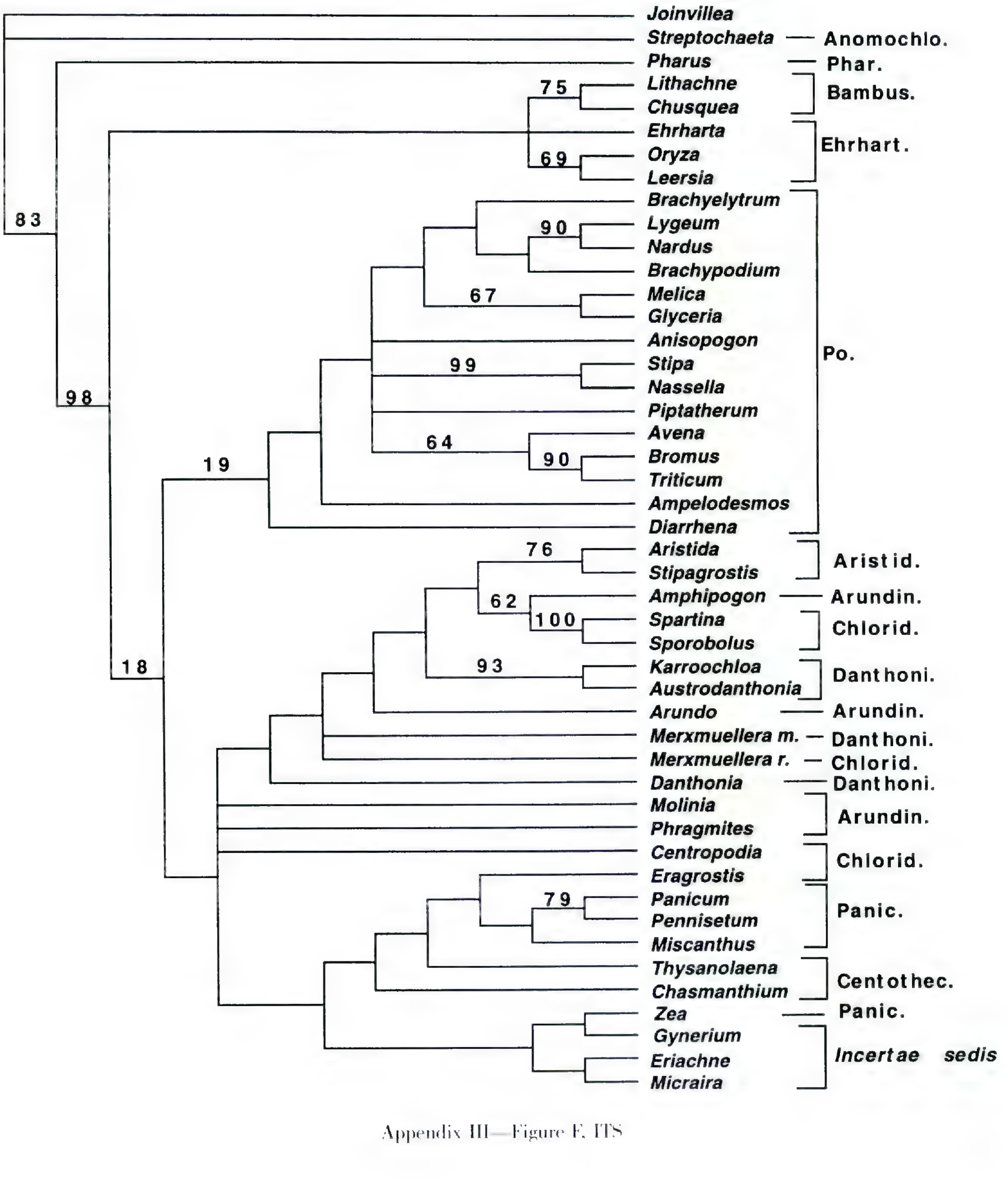
Appendix III—Figure C, *rbc*L



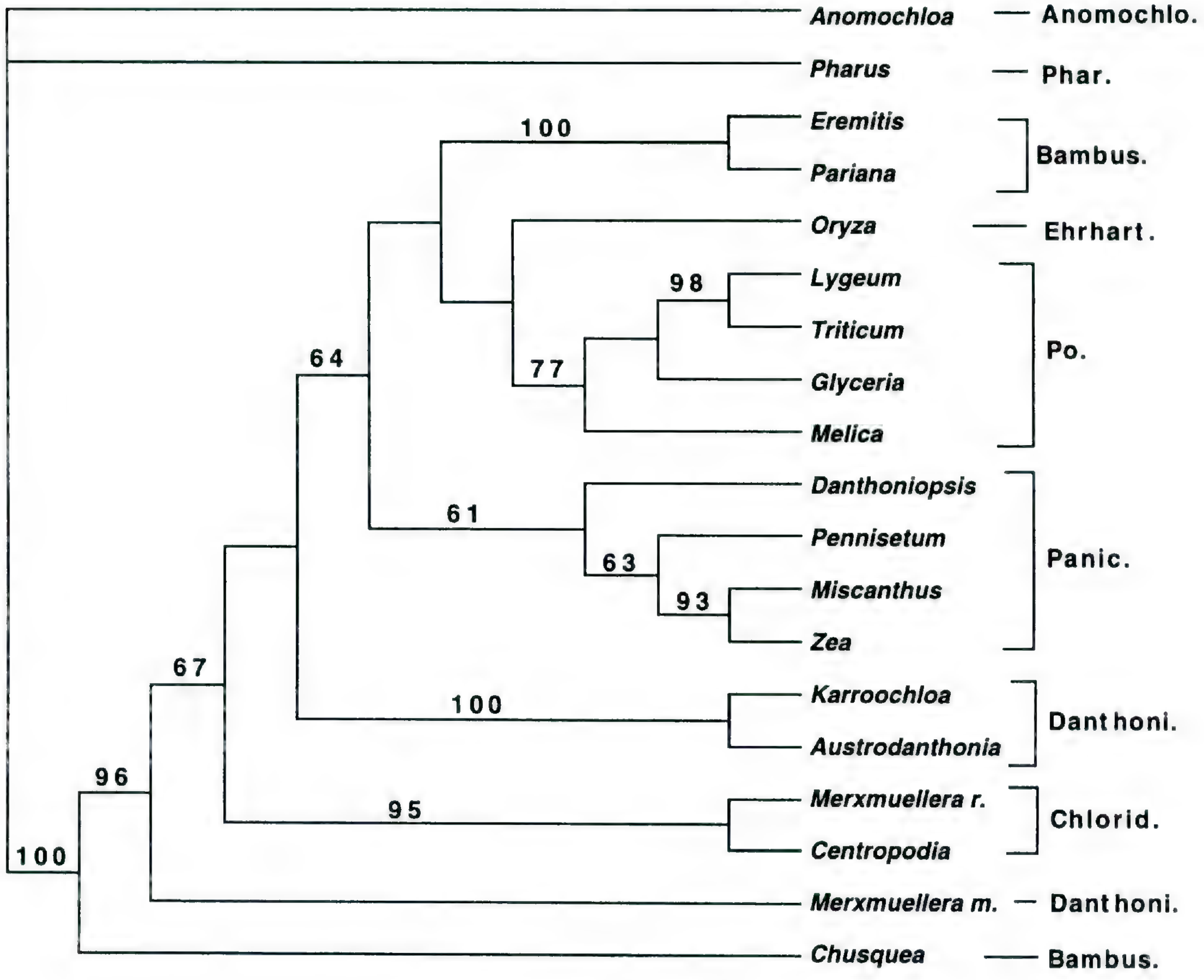
Appendix III—Figure D. *rpo*C2



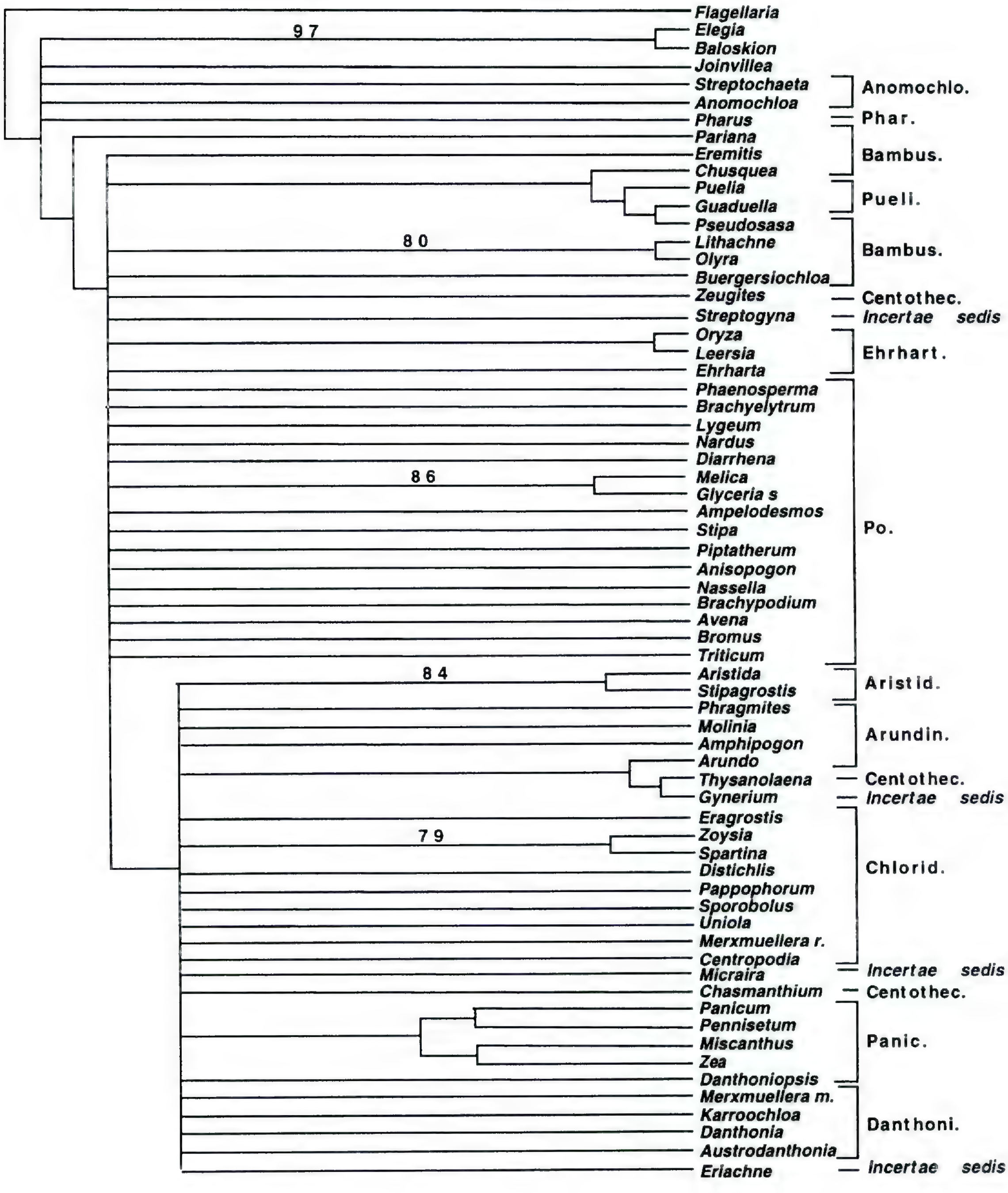
Appendix III—Figure E, phy B



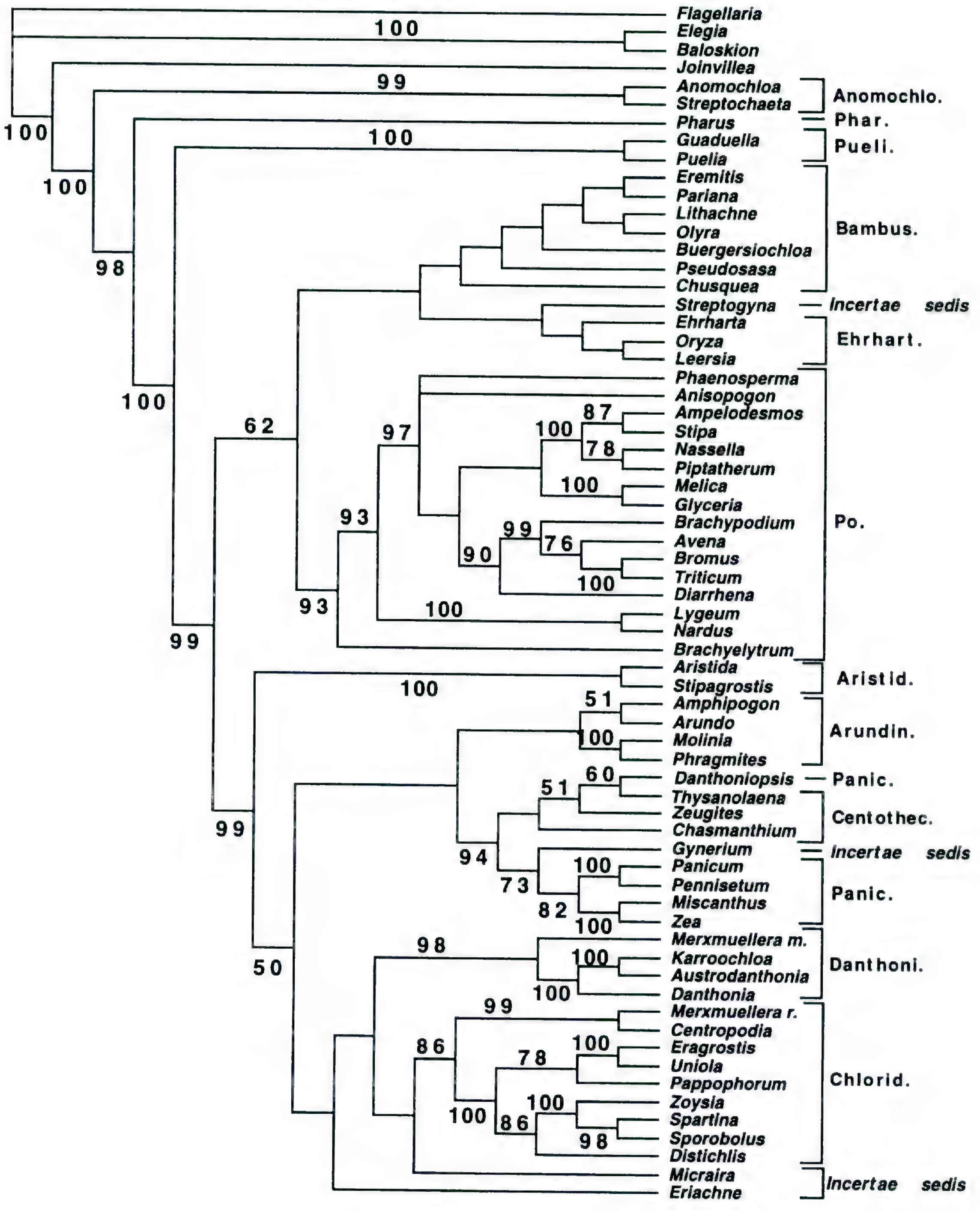
Appendix III—Figure F. ITS



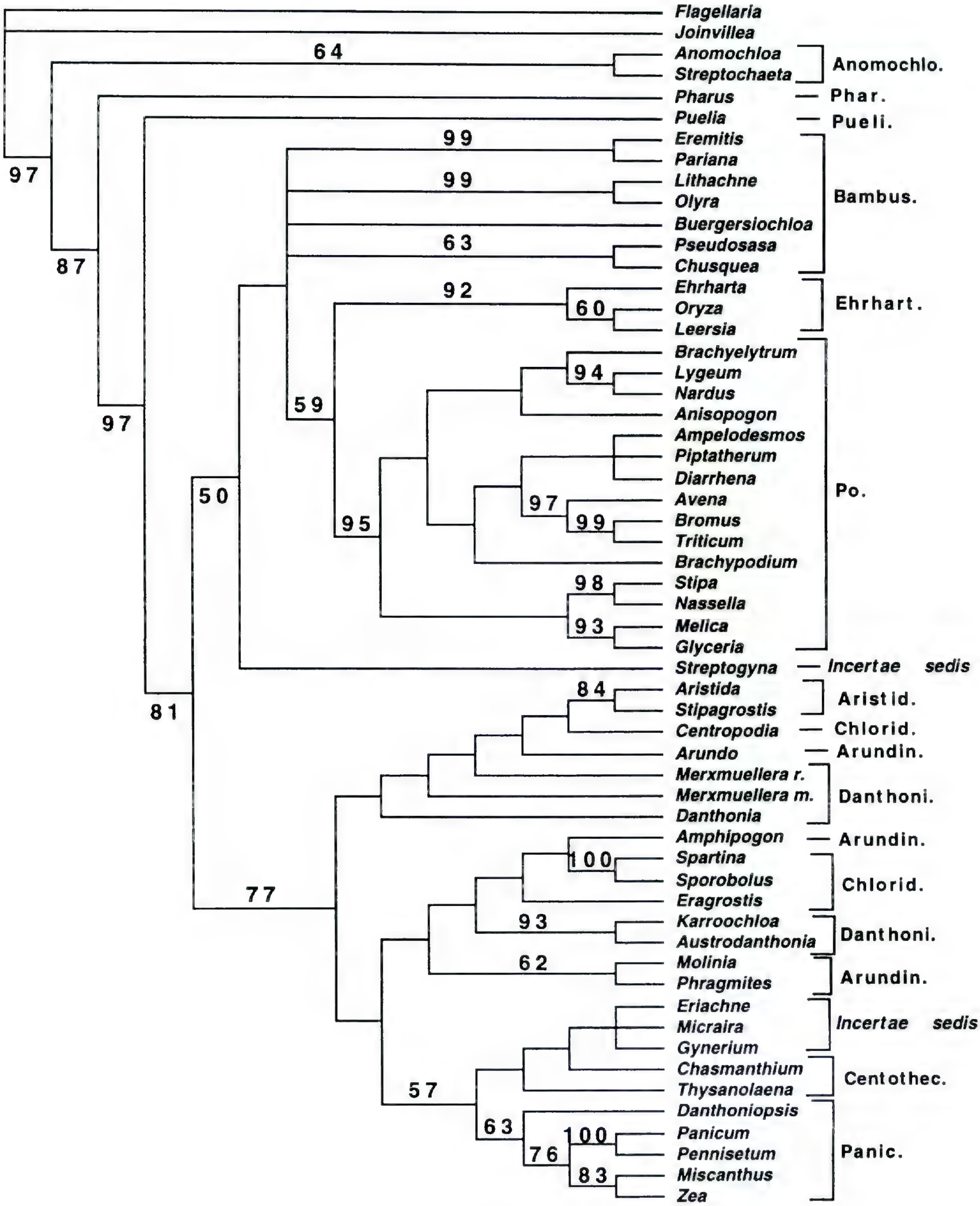
Appendix III—Figure G, GBSSI



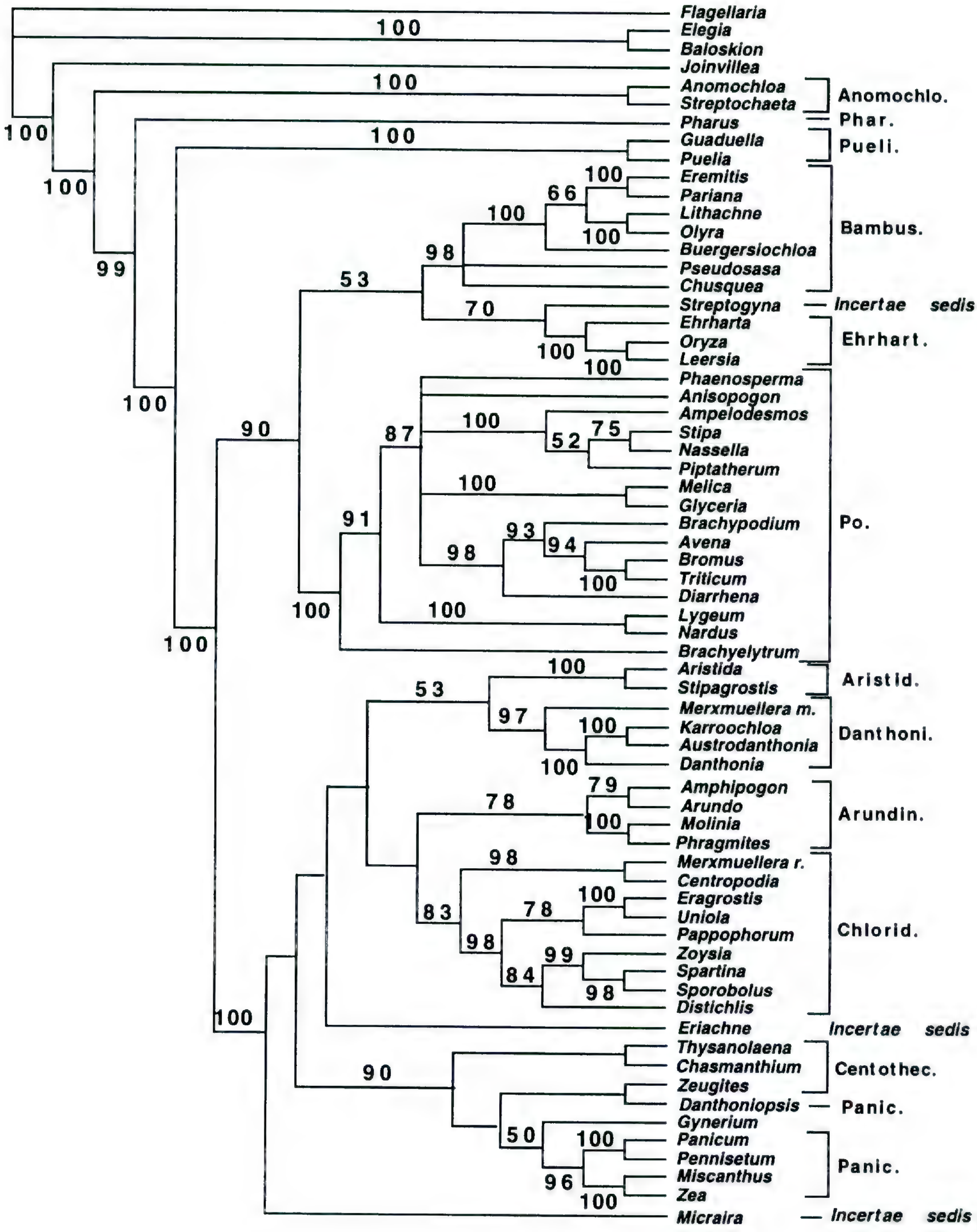
Appendix III—Figure H, structural



Appendix III—Figure 1. all chloroplast



Appendix III—Figure J. all nuclear



Appendix III—Figure K, all molecular

Appendix IV. Notes on morphological characters.

In this section the structural characters are defined and/or discussed, numbered as in Table 1, and their distribution on the most parsimonious tree is outlined. The behavior of each character on the most parsimonious tree is signified by a series of three numbers (number of steps, CI, and RI); p/a refers to presence/absence of a character.

Culm

1 (4 steps, CI = 0.25, RI = 0.25). Perennating woody culms p/a: Highly lignified, perennial culms are absent among outgroups (except for the score of "uncertain" in *Flagellaria*) and in most grasses. While most, if not all, grasses produce some lignin in their culms, the distinction between "woody" and "herbaceous" is usually easy to draw, and our scoring was based on this qualitative criterion. Of the taxa in this analysis, presence is an unambiguous and unreversed synapomorphy of Bambuseae, and an autapomorphy of *Arundo*, *Thysanolaena*, and *Gynerium*; *Phragmites* is scored as intermediate.

2 (14 steps, CI = 0.07, RI = 0.18). Hollow culms p/a: This character is variable in the grasses (14 steps), and polymorphic in at least five of the sampled genera, and many additional ones. Occurrence of a small pore was scored as intermediate. Solid culms are uncommon and scattered in occurrence in the early-diverging lineages and the BEP Clade, but are frequent in the PACCAD Clade, where they are often associated with C_4 photosynthesis. There are no unambiguous synapomorphies in this analysis and the character is highly homoplasious globally, but, as is well known among grass systematists, for many small groups of genera and species one state or the other of this character likely is a synapomorphy.

Leaf

If the leaf blade is absent, characters 15 and 16 are considered inapplicable, but characters 3, 4, and 5 are scored.

3 (3 steps, CI = 0.33, RI = 0.33). Leaf sheath margins free/fused: Fused margins are an unreversed synapomorphy of Meliceae, and autapomorphies of *Flagellaria* and *Bromus*. Fused sheaths are frequent in Poeae and Aveneae and may provide a tribal or more local synapomorphy, or may be plesiomorphic. Sampling outside the grasses would help establish the point of origin of the free leaf sheath.

4 (5 steps, CI = 0.20, RI = 0.76). Adaxial ligule type: The membranous ligule is the most common state in the sample. Transformation to a fringe of hairs is an unreversed synapomorphy of Anomochlooideae, and a synapomorphy of the clade of *Eriachne* plus its sister group (a set of four subfamilies, Aristidoideae, Danthonioideae, Arundinoideae, and Chloridoideae), although the character reverses multiple times within this group. The ligule as a fringe of hairs also is an autapomorphy of *Danthoniopsis*.

5 (4 steps, CI = 0.25, RI = 0.25). Abaxial ligule p/a: Most grasses lack an abaxial ligule. Presence is an unreversed synapomorphy of Bambuseae, and an autapomorphy of *Puelia*, *Streptogyna*, and *Thysanolaena*. Abaxial ligules occur sporadically in the PACCAD Clade, and are known in a few Pooideae.

6 (1 step, CI = 1.0, RI = 1.0). Leaf blade p/a: All sampled species have a leaf blade, except in Restionaceae, where loss of the blade is a synapomorphy. A few species in some grass genera, such as *Ehrharta*, lack a developed blade, but these are clearly interpretable as losses.

7 (4 steps, CI = 0.25, RI = 0.80). Pseudopetiole p/a: The pseudopetiole is a constriction at the base of the leaf blade. Both states occur in the grasses and in the outgroups. Loss of the pseudopetiole is a synapomorphy of the clade that includes all grasses except Anomochlooideae, Pharoideae, and Puelioideae (i.e., the BEP + PACCAD clade). In the present cladogram, the pseudopetiole is interpreted as secondarily gained in Bambusoideae, *Thysanolaena* + *Zeugites*, and *Phaenosperma*.

Spikelet

8 (1 step, CI = 1.0, RI = 1.0). Floret p/a: The floret was defined for the morphological matrix as a unit of the grass inflorescence consisting of a subtending bract (= lemma) enclosing a short axillary axis bearing a flower, the first appendage of which is an adaxial, usually two-keeled bract (= palea). The floret is present only in grasses, but not in all grasses. Of the taxa included in this analysis, the floret is regarded as absent in *Streptochaeta* and of undetermined status in *Anomochloa*, based on the uncertain homologies of their floral bracts and the lack of an identifiable palea (Judziewicz & Soderstrom, 1989; Soreng & Davis, 1998). These two genera have flowers subtended by well-developed bracts, but not in any configuration that can be compared directly to the above definition. Gain of the floret is interpreted as a synapomorphy of the clade of all grasses except Anomochlooideae. Within the spikelet clade, the palea is absent in a number of taxa, including some species of *Agrostis*, Andropogoneae, and, in this analysis, *Zoysia*. All of these taxa, however, have an identifiable lemma and other conveners have paleas. Following a strict definition of the floret, *Zoysia* was scored as polymorphic for this character, although the phylogenetic context shows that a complete floret was present ancestrally.

9 (2 steps, CI = 0.50, RI = 0.50). Spikelet pairs: Spikelet pairs are infrequent in the grasses, and their origin is a synapomorphy of Andropogoneae in this analysis (but note that *Danthoniopsis* is regarded as polymorphic), although spikelet pairs are also found in some Paniceae. Their presence may also be an autapomorphy of *Pharus*, but since Anomochlooideae and non-grasses are not scored for this character, the placement of this transformation is ambiguous (i.e., paired spikelets could be interpreted as plesiomorphic among the floret-bearing grasses). Developmentally, spikelet pairing appears to occur in the same way wherever it appears in the Panicoideae (LeRoux & Kellogg, unpublished obs.), but developmental studies have not been done on *Pharus*, so spikelet pairing is an inference based on adult morphology alone.

10 (4 steps, CI = 0.25, RI = 0.0). Pedicel p/a: The pedicel is present in the earliest-diverging grass lineages that have spikelets. Multiple losses occur, but only the autapomorphic loss in *Triticum* can be placed unambiguously. Loss of the pedicel may be a synapomorphy of *Lygeum* + *Nardus*, but this is ambiguous because this character is scored as ambiguous for *Lygeum*.

11 (7 steps, CI = 0.14, RI = 0.45). Proximal female-sterile florets: Presence of proximal female-sterile florets is interpreted as an unreversed synapomorphy of Puelioideae and of Panicoideae (including *Danthoniopsis*, excluding *Gynerium*), and is a potential synapomorphy for Ehrhartoideae except that this is coded as ambiguous for *Leersia* and *Oryza*. Multiple origins occur elsewhere as

autapomorphies, e.g., in the traditional Aveneae (including Phalarideae), *Phragmites*, *Chasmanthium*, *Uniola*, and *Chusquea*, and there are no unambiguous losses once such florets are gained. As noted in the discussion on spikelets, some proximal female-sterile florets may be homologous to glumes, as in Ehrhartoideae or some Bambuseae, whereas others, as in Panicoideae, are clearly derived from reduction of fertile florets.

12 (13 steps, CI = 0.07, RI = 0.47). Number of female-fertile florets per female-fertile spikelet: The plesiomorphic state among the grasses is one. Increase in the number of female-fertile florets has occurred multiple times, but the placement of these changes is ambiguous, in part because five genera are scored as polymorphic. The only unambiguous synapomorphic reduction from multiple female-fertile florets to one is for the the clade of *Zoysia*, *Spartina*, and *Sporobolus*, although this may not hold up when sampling density is increased. Among unsampled taxa, there are numerous additional transformations to one floret.

13 (12 steps, CI = 0.08, RI = 0.52). Awn or mucro p/a: There are multiple origins of awns and mucros on the lemma, mostly of ambiguous placement. Approximately half the genera of the family have awns, so this is another example of a locally useful but globally highly homoplasious character. In *Streptochaeta*, eleven of the twelve bracts lack awns, whereas one (bract VI) has an awn (Judziewicz & Soderstrom, 1989). These bracts have been variously interpreted (Soderstrom, 1981), but the ones most closely associated with the flower lack awns, so interpretation of the single awned bract as a lemma is doubtful, and *Streptochaeta* consequently is scored as ambiguous for this character.

14 (4 steps, CI = 0.50, RI = 0.50). Number of awns present: This character is scored for taxa that are polymorphic for character 13, but it is inapplicable when character 13 is scored as state 0 or of questionable homology. *Danthonia* and *Austrodanthonia* are scored as state 3 to reflect the basic pattern in these taxa, in which the lemmas have nine veins, with the central vein forming a median, usually articulated or cork-screwed, awn, and the third vein out from the median on each side forms a hair-like awn or seta at the apex of its respective lateral lemma lobe. This seta varies from an acute lobe to a long hair-like extension. Occasionally the lateral lobes are fused with each other, and presumably with the base of the central awn. From the basic pattern all kinds of deviations occur, including fusion of the lateral lobes, loss of the setae, loss of the median awn, and fusion of the lateral lobes with the median awn still present as a stout mucro. In this analysis, presence of three awns is an unreversed synapomorphy of the clade of Aristidoideae + Danthonioideae, but this state also occurs in *Amphipogon* and *Anisopogon* and in other genera not sampled here such as *Plectrachne*, *Triodia*, and *Pentaschistis*, among others. Presence of numerous awns is an autapomorphy of *Pappophorum*.

15 (7 steps, CI = 0.28, RI = 0.44). Awn attachment: This character is scored for taxa that are polymorphic for character 13, but it is inapplicable when character 13 is scored as 0 or of questionable homology. Awn attachment at the apex of the lemma is the most common and widespread state of this character. Attachment in a sinus is an unreversed synapomorphy of Danthonioideae (although it appears to be attached at the apex of the lemma in some instances because of fusion of the lateral lobes with the base of the awn or loss of the lateral lobes; Linder & Verboom, 1996; Linder & Davidse, 1997) and of *Merxmuellera rangei* + *Centropodia*. This state also occurs in *Streptogyna*, *Anisopogon*, *Bromus*, and some Panicoideae. Awn attachment on the back of the lemma is widespread in Aveneae; here it is an autapomorphy of *Avena*, the only taxon from the tribe in this analysis. Among unsampled grasses, dorsal attachment is known from one genus of Meliceae and a few genera of the PACCAD Clade including *Arthraxon*.

16 (6 steps, CI = 0.16, RI = 0.44). Disarticulation above glumes: Glumes are considered to be the two empty bracts subtending the spikelet, and glumes across the spikelet clade were assumed to be homologous even though there is disagreement on this point (see Discussion). This character is not scored in Anomochlooideae or in the non-grass outgroups, which lack spikelets and therefore glumes, but the presence of this type of disarticulation in at least some members of the Pharoideae (e.g., *Leptaspis* and *Pharus*; Soderstrom et al., 1987), and in both genera of the Puelioideae, argue that this type of disarticulation is plesiomorphic in the Spikelet Clade. Synapomorphic loss of disarticulation above the glumes occurs in *Pariana* + *Eremitis* and in the clade within Panicoideae that consists of Paniceae + Andropogoneae. Additional losses, all autapomorphic or potentially so, occur in *Zoysia*, *Spartina*, *Phaenosperma*, and *Melica*.

17 (6 steps, CI = 0.16, RI = 0.37). Germination flap: The germination flap, a small flap of tissue at the base of the lemma through which the germinating embryo grows, is derived independently within the PACCAD and BEP Clades. There are unambiguous independent synapomorphic gains of the germination flap in Aristidoideae and Olyreae; germination flaps also are present in some Panicoideae (but placement of the transformation is ambiguous although no Andropogoneae have germination flaps), and the character is also autapomorphic in *Oryza* and in *Amphipogon*.

Flower

Characters 19–22 are scored as inapplicable for taxa that lack lodicules.

18 (4 steps, CI = 0.25, RI = 0.70). Lodicule p/a: Lodicules, as organs that become turgid at anthesis and force open the flower, occur in most grasses and are not present outside the grasses. The evidence that lodicules are modified tepals is not universally accepted. *Anomochloa*, *Streptochaeta*, and non-grasses were scored "uncertain homology" by Soreng and Davis (1998), but these two genera are scored as lacking lodicules in the present analysis. In the Pharoideae, lodicules are present or absent in the male spikelets and lacking in female spikelets (Clark & Judziewicz, 1996). *Pharus* is scored here as having lodicules. Thus, the first unambiguous occurrence of lodicules is in the clade that consists of all grasses except Anomochlooideae. There is an unambiguous synapomorphic loss in *Lygeum* + *Nardus* and another in *Zoysia* + *Spartina* + *Sporobolus*, plus an autapomorphic loss in *Micraira*. Among unsampled grasses, 84 taxa lack lodicules; among these, 60 belong to the PACCAD Clade, and 13 to the Pooideae (Watson & Dallwitz, 1992). Lodicules (whatever their origin) might be plesiomorphic for the family, and then lost in the Anomochlooideae.

19 (5 steps, CI = 0.20, RI = 0.71). Number of lodicules: Three lodicules are present at the point of first unambiguous occurrence of lodicules (see char. 18), and this plesiomorphy is retained in Pharoideae and Puelioideae.

Bambusoideae and *Streptogyna* also have three lodicules, while Ehrhartoideae have two. Transformation to two lodicules may be a synapomorphy of Ehrhartoideae, but cannot be inferred unambiguously because the earliest-diverging lineages of Pooideae also have two lodicules, as do all taxa of the PACCAD Clade that have been scored. Thus two lodicules may be a synapomorphy of the BEP + PACCAD group, with a reversal to three lodicules in the Bambusoideae/Ehrhartoideae group, and re-reversal to two lodicules in the Ehrhartoideae. Alternatively, two lodicules might have been retained in Ehrhartoideae (following the transformation from three to two in the origin of the BEP + PACCAD group) while *Streptogyna* and Bambusoideae independently experienced reversals to three; still other transformation sequences also are possible. Within Pooideae, an unambiguous transformation from two lodicules to three is a synapomorphy of the clade that includes *Anisopogon*, *Phaenosperma*, *Ampelodesmos*, and Stipeae, but within this group there is yet another unambiguous transformation back to two lodicules in *Nassella* (while *Stipa* is polymorphic).

20 (uninformative). Fusion of anterior pair of lodicules: Lodicules are unfused at the first point at which they are unambiguously present (see char. 18), and in almost all grasses. The anterior two are fused in *Melica*, but the character is polymorphic in *Glyceria*, so the transformation may be a synapomorphy of this pair of genera, but the precise placement is ambiguous. Elsewhere, *Molinia* also is polymorphic.

21 (5 steps, CI = 0.20, RI = 0.82). Distally membranous portion of lodicule: The earliest lodicules apparently had a distally membranous portion (see char. 18), and this state is retained in early-diverging lineages. Within the BEP Clade, loss of this membranous portion is an unreversed synapomorphy of Meliceae. Most elements of the PACCAD Clade lack a distally membranous portion of the lodicule, including various early-diverging lineages, but *Micraira*, because it lacks lodicules, is not scored for this character. Thus, transformation to this state may be a synapomorphy of the entire PACCAD Clade, or of the subset that includes all members except *Micraira*. Within the PACCAD Clade, a distally membranous lodicule in Aristidoideae, *Gynerium*, and *Merxmuellera macowanii* implies at least three additional steps in this character, including either three independent transformations to this lodicule type, or two independent gains including one in the common ancestry of Aristidoideae and Danthonioideae, followed by a loss in the ancestor of *Danthonia*, *Karroochloa*, and *Austrodanthonia*. Polymorphism in *Panicum* and *Leersia* implies two additional transformations, a gain of the membranous portion of the lodicule within the former, and a loss within the latter.

22 (3 steps, CI = 0.33, RI = 0.83). Lodicule vascularization: Lodicules were originally vascularized (see char. 18). In the BEP Clade, a single unreversed loss of vascularization is inferred within Pooideae, after divergence of *Brachyelytrum* from the rest of the subfamily, but absence of lodicules in the *Lygeum* + *Nardus* group means that this transformation could have occurred before or after divergence of this group from the rest of the Pooideae. In the PACCAD Clade, an independent transformation to faint vascularization is an autapomorphy of *Amphipogon*. The state also occurs in *Thysanolaena*, but the association of the latter with *Zeugites*, which is not scored for this character, prevents an unambiguous placement of that transformation.

23 (5 steps, CI = 0.20, RI = 0.66). Posterior stamen of the inner whorl: The posterior stamen of the inner whorl is present in all outgroups and among the earliest-diverging lineages in the grasses, so its presence is a plesiomorphy for the grasses. Loss of this stamen is a synapomorphy of the BEP + PACCAD clade, but it is regained at least three (possibly four) times in *Ehrharta*, *Oryza* (but not *Leersia*), *Pseudosasa*, and *Pariana*. The absence of this stamen in *Leersia*, coupled with its presence in *Oryza* and *Ehrharta*, is equally consistent with independent gains in the latter two, or a gain in the ancestor of Ehrhartoideae followed by a secondary loss in *Leersia*. This stamen is not gained elsewhere in the family.

24 (6 steps, CI = 0.16, RI = 0.54). Anterior stamen pair of the inner whorl: Except for the loss of this pair in *Anomochloa*, while the posterior stamen is retained, the distributions of states of these two characters (23 and 24) are identical. Thus, as with the posterior stamen of the inner whorl, this stamen pair is unambiguously interpreted as plesiomorphically present at the point of origin of the grasses, lost as a synapomorphy of the BEP + PACCAD clade, and regained three or four times in the Bambusoideae/Ehrhartoideae, possibly with a secondary loss in *Leersia*.

25 (8 steps, CI = 0.12, RI = 0.12). Anterior stamen of outer whorl: This stamen, though absent in Restionaceae, is present in *Flagellaria*, *Joinvillea*, and all early-diverging grass lineages, and thus is plesiomorphically present within the grasses, and lost independently in Restionaceae and various grass lineages. There are seven autapomorphic losses within the grasses (in *Streptogyna*, *Leersia*, *Eremitis*, *Glyceria*, *Piptatherum*, *Micraira*, and *Gynerium*), plus polymorphisms in *Buergersiochloa*, *Diarrhena*, *Eriachne*, *Zoysia*, *Sporobolus*, and *Thysanolaena*. The loss in Restionaceae is the only unambiguous synapomorphic loss in the taxon sample.

26 (2 steps, CI = 0.50, RI = 0.50). Posterior stamen pair of outer whorl: This stamen pair, like the anterior stamen of the outer whorl, is present in all early-diverging grass lineages and in all outgroups except Restionaceae. Thus, like the anterior stamen and like all three stamens of the inner whorl, this pair is plesiomorphically present in the study sample as well as in grasses, and as with the anterior stamen of the outer whorl, all absences are interpreted as losses. Except for a few unscored taxa within the grasses, plus an autapomorphic loss in *Chasmanthium* and a polymorphism in *Sporobolus*, presence of this pair of stamens is constant within the grasses.

27 (1 step, CI = 1.0, RI = 1.0). Anthers tetrasporangiate, dithecal, vs. bisporangiate, monothecal: The presence of tetrasporangiate, dithecal anthers is interpreted as a plesiomorphy of the entire taxon sample, and of the grasses. The only transformation to bisporangiate, monothecal anthers is an unambiguous synapomorphy of Restionaceae.

28 (12 steps, CI = 0.08, RI = 0.31). Fusion of styles: The presence of one style (see char. 29) can be interpreted as either a fusion or reduction in number, so taxa scored with state 1 of character 29 are scored as ambiguous for character 28. Unfused styles in *Flagellaria* and both representatives of Restionaceae, and fused styles in *Streptochaeta*, *Pharus*, and *Puelia* together suggest that transformation to the fused state occurred near the origin of the grasses, but polymorphism in *Joinvillea* and the unknown state in *Anomochloa* prevent unambiguous placement of this transformation. Reversal to unfused styles is interpreted as an autapomorphy in *Guaduella*, and as unreversed synapomorphies (i.e., lacking secondary reversion to

fused styles) of Pooideae and Ehrhartoideae (except for polymorphism in *Oryza*). Within the PACCAD Clade, fused styles appear to be plesiomorphic, with one or more transformations to the nonfused state, and multiple reversals to the fused state (e.g., in *Phragmites*, *Uniola*, and *Spartina*).

29 (6 steps, CI = 0.33, RI = 0.50). Number of stigmas: The plesiomorphic state for the grasses, as well as for the entire taxon set, is three, with autapomorphic transformations to two in *Baloskion* and, among earliest-diverging grass lineages, to one in *Anomochloa*. Transformation to two stigmas is a synapomorphy of the clade that includes all grasses except Anomochlooideae and Pharoideae (i.e., the Bistigmatic Clade), and there are additional transformations to one in *Eremitis* and in *Lygeum* + *Nardus*, and to three in *Pseudosasa*. Another transformation to one appears to occur in *Eremitis*, but this is the result of a miscoding, as there are actually two stigmas in *Eremitis* (V. Hollowell, pers. comm.). *Puelia* is actually polymorphic for this character, having two or three stigmas (the species in this analysis has two). Other polymorphisms (see data table) signify additional transformations. All members of the PACCAD Clade have two stigmas.

30 (8 steps, CI = 0.25, RI = 0.50). Highest order of stigmatic branching: Only one order of stigmatic branching occurs in *Flagellaria*, *Joinvillea*, Anomochlooideae, and Pharoideae, and this state therefore appears to be plesiomorphic for the grasses and for the entire taxon sample, with the various states in Restionaceae interpretable as apomorphic within that family. Transformation to two orders of branching appears to be a synapomorphy for the clade that includes all grasses except Anomochlooideae and Pharoideae (the Bistigmatic Clade), and this state, once established, is constant in Puelioideae and nearly so in the PACCAD Clade (exceptions being the presence of state one in *Zea*, and polymorphism in *Sporobolus*). Within the Bambusoideae/Ehrhartoideae, transformation to state one is an autapomorphy of *Streptogyna* and a synapomorphy of *Pariana* + *Eremitis*, while a transformation to state 3 is an autapomorphy of *Leersia*, and polymorphisms occur in unsampled Olyreae. Within the Pooideae, there is an autapomorphic transformation to state one in *Nardus*, a synapomorphic transformation to state three in Meliceae, and polymorphism in *Phaenosperma*.

31 (3 steps, CI = 0.66, RI = 0.0). Number of locules: All grasses have one locule with one ovule, while three locules, each with one ovule, are found in *Flagellaria* and *Joinvillea*, although there is a strong tendency to abort one or two of the ovules in both of these genera. A transformation from three locules to one may be a synapomorphy of the grasses. This transition has also occurred in Restionaceae, where the basal condition is three locules with numerous reductions to a single functioning locule, as in *Elegia* in our sample (Linder, 1992a, b). Although not sampled in this study, Anarthriaceae have three locules each with one ovule, but the fruit is 1-seeded, and Ecdeiocoleaceae have two locules, each with one ovule, and the fruit is 1–2-seeded. Thus, reduction in both number of locules and number of ovules that develop in the fruit are common in the Poales; Centrolepidaceae, however, have uniloculate, uniovulate ovaries that are apparently monocarpellary (Dahlgren et al., 1985).

Embryogeny

32 (1 step, CI = 1.0, RI = 1.0). Haustorial synergids p/a: Data are unavailable for many taxa, but haustorial synergids are observed to be present only in the four sampled taxa of the Danthonioideae, and absent in all other taxa that have been examined (Verboom et al., 1994). Thus, presence of haustorial synergids is an unambiguous and unreversed synapomorphy of Danthonioideae, but continued investigation is warranted.

Fruit and Embryo

Characters 35–38 describe features of the typical grass-type embryo. They are inapplicable for non-grass genera, which lack the grass-type embryo (i.e., state 0 of char. 34). Data sources include those listed by Soreng and Davis (1998) plus Klak (unpublished). These characters can be difficult to score, and the literature contains conflicting reports for some taxa.

33 (5 steps, CI = 0.20, RI = 0.76). Hilum shape: This character is recognized as a feature of the caryopsis, and thus is inapplicable for non-grasses. Among grasses, taxa with a short hilum less than one-third the length of the grain are scored as ambiguous. All groups except the PACCAD Clade have a long hilum that is greater than one-third the length of the grain in our sample. This may thus be a synapomorphy of grasses (i.e., the plesiomorphic state for the caryopsis), but because the character is treated as inapplicable outside the grasses there is no observed transformation at the origin of the family. Nonlinear hila are reported from the Olyreae and the Poeae/Aveneae in the BEP Clade. There are five character transformations within the PACCAD Clade, but the placements of two of these are ambiguous. All Centothecoideae and most Panicoideae have a nonlinear hilum that is less than ⅓ the length of the grain, but the hilum is long-linear and greater than ⅓ the length of the grain in *Danthoniopsis*, and this is interpreted as one origin of this state in the common ancestor of the clade, followed by a reversal in *Danthoniopsis*. Elsewhere in the PACCAD Clade, the only unambiguous transformation of this character is as a synapomorphy of *Karroochloa* + *Austrodanthonia*, a subset of Danthonioideae. This state also occurs in all taxa of Arundinoideae and Chloridoideae for which there are observations, except in *Molinia*. Thus, it is interpretable either as having arisen twice (once in *Amphipogon* + *Arundo*, and once in Chloridoideae), or as a synapomorphy of Arundinoideae + Chloridoideae, with reversion to a long-linear hilum in *Molinia* or in *Molinia* + *Phragmites* (not scored for *Phragmites*).

34 (1 step, CI = 1.0, RI = 1.0). Grass-type embryo p/a: The grass-type embryo is lateral, peripheral to the endosperm, and differentiated in fruit (Reeder, 1957; Cronquist, 1981; Sendulsky et al., 1987). This embryo type is absent outside the grass family and present in all grasses for which observations are available (unobserved in *Merxmuellera rangei*), and thus interpreted as an unambiguous and unreversed synapomorphy of the grasses.

35 (7 steps, CI = 0.14, RI = 0.66). Epiblast p/a: Because this character is inapplicable for non-grasses, its origin is not unambiguously fixed, but available data for the earliest-diverging lineages within the grasses (except *Streptochaeta*) indicate presence of an epiblast, and thus presence is interpretable as a plesiomorphy within the family. Loss of the epiblast is an unambiguous synapomorphy of the PACCAD Clade, but there are also three autapomorphic losses outside of the PACCAD Clade in *Streptochaeta*, *Ehrharta*, and *Bromus*, plus a polymorphism in *Brachypodium*. Within the PACCAD Clade the epiblast is secondarily gained in the Centothecoideae (but

there is no observation for *Thysanolaena*) and is a synapomorphy for either the Chloridoideae or a subset of that clade. The precise point of origin within the Chloridoideae is ambiguous because there is no observation for *Merxmuellera rangei* or *Centropodia*. Within the Chloridoideae the epiblast is lost in *Uniola*. This character is highly homoplasious within the family.

36 (3 steps, CI = 0.33, RI = 0.81). Embryo scutellar tail p/a: The scutellar tail is present in Anomochlooideae and Pharoideae, throughout the PACCAD Clade (wherever observations are available) and in most taxa of the Bambusoid/Ehrhartoid clade, absent in most Pooideae, and unobserved in Puelioideae. Despite the variation just described, plus the inapplicability of this character outside the grasses and the absence of data for some critical taxa, the balance of evidence suggests that the scutellar tail is a plesiomorphy of the grasses. Within the Bambusoid/Ehrhartoid clade, loss of the scutellar tail is unambiguously interpreted as an unreversed synapomorphy of Oryzeae. A more complex pattern is present in Pooideae, where the scutellar tail is usually absent, but is present in *Phaenosperma*, and there are polymorphisms or conflicting reports for *Brachyelytrum*, *Diarrhena*, and *Avena*. These polymorphisms in the matrix prevent unambiguous optimization of the character in Pooideae. Loss of the scutellar tail may be a synapomorphy of Pooideae or of all Pooideae except *Brachyelytrum*; presence of the scutellar tail in *Phaenosperma* may be either a unique reversal or a synapomorphy for *Phaenosperma* and *Anisopogon* (which is not scored).

37 (2 steps, CI = 0.50, RI = 0.95). Embryo mesocotyl internode, negligible vs. elongated: The embryo mesocotyl internode is negligible in length in all early-diverging grass lineages for which scores are available (e.g., Anomochlooideae, Pharoideae), so although this character is inapplicable for the non-grass outgroups the internode is likely to have been negligible at the origin of the grass-type embryo. All taxa that have been scored in the PACCAD Clade have an elongated internode. Transformation from a negligible to an elongated internode is either a synapomorphy of the entire PACCAD Clade or of the entire clade except *Micraira*, for which no observation is available.

38 (4 steps, CI = 0.25, RI = 0.85). Embryonic leaf margins meeting vs. overlapping. The margins of the embryonic leaf meet in *Anomochloa* but overlap in *Streptochaeta*, *Pharus* (unobserved in Puelioideae), and early-diverging lineages of both the BEP and PACCAD Clades. Thus, although the character is inapplicable in the non-grass outgroups, overlapping leaf margins are plesiomorphic at the origin of the grass-type embryo, with an autapomorphic transformation to margins meeting in *Anomochloa*, and with parallel synapomorphic transformations in both major lineages. Margins are overlapping in all observed taxa in the bambusoid/ehrhartoid alliance, as well as in *Brachyelytrum* and *Phaenosperma* of the Pooideae (also, *Diarrhena* is polymorphic). Given this distribution, transformation to the margins meeting is interpreted as a synapomorphy of all Pooideae except *Brachyelytrum*, with a reversal either in *Phaenosperma* or in the ancestor of *Phaenosperma* and *Anisopogon* (there is no observation for the latter). All observations for Panicoideae and Centothecoideae, plus *Eriachne*, are of leaf margins overlapping, while all observations for the other four subfamilies of the PACCAD Clade are of leaf margins meeting. Thus, transformation to the latter state is an unambiguous and unreversed synapomorphy of the sister group of *Eriachne*, the clade that includes Aristidoideae, Danthonioideae, Arundinoideae, and Chloridoideae.

39 (uninformative). Endosperm lipid p/a: Observations are unavailable for several taxa. Of those taxa that are scored, including *Baloskion*, only *Avena* has lipid in the endosperm. Thus, absence of lipid in the endosperm is plesiomorphic for the grass family and for the taxon set as a whole, with the presence of lipid in *Avena* an autapomorphy. Among unsampled grasses, all reports of endosperm lipid are from the Poeae–Aveneae. Liquid and semi-liquid endosperm are indicative of the presence of lipid, but "semi-solid" and solid states do not imply absence of lipid (Terrell, 1971; Rosengurtt et al., 1972).

40 (12 steps, CI = 0.33, RI = 0.20). Starch grain syndromes: Scoring here follows Tateoka's (1962) classification with one exception. Tateoka scored *Brachyelytrum* as having simple *Panicum*-type grains, but emphasized a major size difference, and we recognize *Brachyelytrum*-type as a separate state (see also Campbell et al., 1986). Watson and Dallwitz (1992) distinguished between starch grains "simple only" (coded here as [034]) or "compound" (coded here as [12]). Polymorphisms, ambiguity of state delimitation, and lack of observations together preclude unambiguous optimizations of many character-state transformations in this multistate character, but some patterns are evident. First, the *Festuca*-type grain (state 1) is present in *Baloskion* (the only non-grass that is scored) and is widespread in early-diverging grass lineages, among which other types are not observed. This pattern suggests that this starch grain syndrome is plesiomorphic for the grass family and for the taxon set as a whole. The *Triticum*-type syndrome (state 0) occurs in most "core" Pooideae (represented here by *Brachypodium*, *Avena*, *Bromus*, and *Triticum*) that are collectively the sister group of *Diarrhena*, and state 0 may be either a synapomorphy of this group (reversed, however, in *Avena*) or a parallelism that arises separately in *Brachypodium* and in the ancestor of *Bromus* + *Triticum*. The *Panicum*-type syndrome (state 3) may be a synapomorphy of Bambuseae, of *Stipa* + *Nassella*, and of all Panicoideae except *Danthoniopsis*, or of the entire PACCAD Clade. If the latter is true, then there is a reversal to the *Festuca*-type in the clade of *Eriachne* plus the set of four subfamilies that is its sister; the *Festuca*-type is also a potential synapomorphy of *Thysanolaena* + *Zeugites*, or an autapomorphy of *Zeugites*. The *Brachyelytrum*-type syndrome occurs in *Phaenosperma* and *Brachyelytrum*. Available information suggests that *Stipa* may also have this state (see Soreng & Davis, 1998).

Seedling

41 (4 steps, CI = 0.25, RI = 0.70). Lamina of first seedling leaf p/a: The lamina of the first seedling leaf is absent in *Flagellaria* and Anomochlooideae, and present in Restionaceae and Pharoideae, while the character is unobserved in other non-grass taxa and other early-diverging lineages within the grasses. Consequently, optimization of this character is ambiguous in this region of the tree. However, presence of the lamina is unambiguously established by the point of divergence of Pharoideae from the lineage that includes most other grasses; it is present in the PACCAD Clade and in Pooideae, but is lost twice within the BEP Clade, as a synapomorphy of Oryzeae and as a synapomorphy of Bambusoideae.

Vegetative Anatomy

42 (uninformative). Differentiation of leaf epidermal cells into long and short cells: Differentiation is absent in

Flagellaria, inapplicable in Restionaceae (blades absent), and present in *Joinvillea* and all grasses (except for a few that are polymorphic). This differentiation is therefore established by the time of divergence of *Joinvillea* from the grasses, but the point of origin is ambiguous.

43 (6 steps, CI = 0.16, RI = 0.72). Multicellular microhairs p/a: Presence of multicellular microhairs on the abaxial surface of the leaf blades in *Joinvillea*, Anomochlooideae, and *Guaduella*, and their absence in Restionaceae, Pharoideae, and *Puelia*, make it difficult to place the origin (or origins) of multicellular microhairs, but they appear to have been present in the common ancestor of *Joinvillea* and the grasses, and are inferred to have been lost independently (among the aforementioned taxa) in Pharoideae and *Puelia*. Multicellular microhairs are universally present within the PACCAD Clade in our sample, except for an autapomorphic loss in *Merxmuellera rangei* and a polymorphism in *Spartina*. The score for *M. macowanii* was inferred from reports for the rest of the genus; Ellis (1981) reported the absence of microhairs on the abaxial epidermis but did not investigate the adaxial epidermis, where they are most likely to occur. We note, however, that multicellular microhairs have not been detected on the abaxial leaf surface in some species of 40 PACCAD genera, but many of these genera are polymorphic for this character (Watson & Dallwitz, 1992). In contrast with their widespread occurrence throughout most of the family, multicellular microhairs occur in only two genera of the Pooideae, *Lygeum* and *Nardus*. Either microhairs were lost in the common ancestor of the subfamily, followed by a secondary gain in the common ancestor of *Lygeum* and *Nardus*, or were lost twice, once in *Brachyelytrum*, the other time in the ancestor of all pooids except *Brachyelytrum*, *Lygeum*, and *Nardus*. Some taxa reported to lack multicellular microhairs on the abaxial surface of the leaf blades may have such hairs on the adaxial leaf surface or elsewhere on the plant, particularly lemmas or lodicules, and more detailed examination should be undertaken to verify this. Only the clade within Pooideae that is the sister of *Lygeum* + *Nardus* lacks multicellular microhairs entirely, but even in this clade, unicellular microhairs have been reported in several genera of Stipeae (Watson & Dallwitz, 1992).

44 (3 steps, CI = 0.33, RI = 0.71). "Chloridoid-type" microhairs p/a: Tateoka et al. (1959) distinguished microhairs with short and wide apical cells as "Chloridoid-type," as they are mainly restricted to subfamily Chloridoideae. This type of hair is contrasted with "panicoid-type" microhairs, which have relatively longer and thin-walled terminal cells and are widespread among non-Chloridoid grasses. This distinction has been recognized in subsequent studies (Johnston & Watson, 1976; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992). Our scoring of presence vs. absence of chloridoid-type microhairs implies that the two types are clearly distinguishable, but in fact there is a continuum of variation between them (Van den Borre, 1994; Van den Borre & Watson, 1994; E. A. Kellogg, unpublished data). In future analyses this character should be considered very carefully, as many taxa may actually be intermediate or polymorphic. This character is inapplicable for taxa scored 0 for character 43. Absence of chloridoid microhairs is plesiomorphic for the family. This type of hair is gained in *Lygeum*, in *Amphipogon*, and as a synapomorphy in Chloridoideae as traditionally circumscribed (i.e., excluding *Centropodia* and *Merxmuellera rangei*). This gain in the Chloridoideae is unreversed, except for a polymorphism in *Eragrostis* (Van den Borre, 1994; Van den Borre & Watson, 1994).

45 (8 steps, CI = 0.12, RI = 0.46). Arm cells p/a: Arm cells are invaginated chlorenchyma cells that, when present, reach their maximum development in the layer of chlorenchyma beneath the upper surface of the leaf. Variation in arm cells in the Poaceae is known but has not been investigated, and only presence/absence was scored for this analysis. Presence in *Anomochloa*, Pharoideae, and Puelioideae (including a polymorphism in *Guaduella*), and absence in *Streptochaeta*, combined with absence outside the Poaceae, together suggest a first occurrence of arm cells near the origin of the family, but optimization of the transformation to arm cells at the point of origin of the family is ambiguous. Placement of additional gains and losses is complicated by a widespread occurrence in the bambusoid and ehrhartoid clades, polymorphy within *Ehrharta* and *Leersia*, total absence in Pooideae, and sporadic occurrence in the PACCAD Clade (in *Thysanolaena*, *Gynerium*, and *Phragmites*). This overall distribution is consistent with a variety of optimizations that imply multiple origins and losses. Some taxa with arm cells lack fusoid cells, and vice versa, but the occurrence of both cell types is correlated with broad leaf blades and the forest habitat.

46 (4 steps, CI = 0.25, RI = 0.76). Fusoid cells p/a: Fusoid cells are large, clear, cigar-shaped empty cells that flank each vascular bundle and can occupy up to 30% of the leaf blade volume. Although their function is still unknown, they appear to form gas spaces rather than liquid spaces and may play some role in photosynthesis (Clark, 1991). Fusoid cells are absent outside the Poales and, among the families sampled in this analysis, are also absent in Flagellariaceae. They are present in *Joinvillea* and all three of the early-diverging lineages within the grasses. Thus, presence of fusoid cells may be a synapomorphy of *Joinvillea* and Poaceae, but the precise point of origin of fusoid cells is ambiguous in the present analysis, in part because the character is scored as inapplicable in Restionaceae. Elsewhere in the grasses, fusoid cells occur only in Bambusoideae and *Streptogyna*. Fusoid cells are absent in Ehrhartoideae, Pooideae, and the PACCAD Clade, and this pattern is interpretable either as a synapomorphic loss in BEP + PACCAD (followed by secondary gain in *Streptogyna* and Bambusoideae), or parallel losses in Ehrhartoideae, Pooideae, and the PACCAD Clade. Fusoid-like cells are known in some Paniceae, but these appear to be derived from laterally extended bundle sheath cells and thus are not homologous to fusoid cells.

Chromosomes

47 (23 steps, CI = 0.34, RI = 0.44). Base chromosome number: This multistate character varies among taxa, is scored as ambiguous in some, and is polymorphic in others, so the positions of most transformations cannot be reconstructed unambiguously. However, the base number $x = 12$ in Pharoideae, Puelioideae, the BEP Clade, and the PACCAD Clade, suggests early establishment of this state. If the base numbers 11 (in *Streptochaeta*) and 18 (in *Joinvillea* and *Anomochloa*) are derived from 12, then $x = 12$ may predate the origin of the grass family, but other reasonable interpretations also are possible. Nevertheless, our data support the interpretation that $x = 12$ was established prior to divergence of Pharoideae and Puelioideae from the BEP + PACCAD lineage, and that this base number was maintained in some sublineages of both the BEP and PACCAD Clades. This number is main-

tained (or re-evolved) in *Streptogyna*, Ehrhartoideae, and some Bambusoideae, and transformations to other base numbers occur within the Bambusoideae (e.g., $x = 11$ in Olyreae). Within Pooideae, the occurrence of $x = 10$ in *Lygeum*, *Diarrhena*, and some Meliceae suggests that this number was established early in the history of this subfamily (with $x = 11$ in *Brachyelytrum* possibly derived from $x = 10$ as well, but also possibly derived directly from $x = 12$ in the common ancestor of Pooideae and the bambusoid/ehrhartoid lineage). $x = 12$ in *Phaenosperma*, *Ampelodesmos*, and some Stipeae is interpreted here as a secondary transformation from $x = 10$, but placement of these lineages as early-diverging groups within Pooideae, and the occurrence of $x = 12$ in other early-diverging lineages, suggests that this state may be a plesiomorphy retained from its original establishment near or before the point of origin of the family. Transformation to $x = 7$ is a synapomorphy of the clade that includes *Brachypodium*, *Avena*, *Bromus*, and *Triticum*, which suggests that all other numbers within the tribes represented by this sample of genera (including $x = 5$ in some *Brachypodium*, and various numbers in Aveneae and Poeae) are derived from $x = 7$. Poeae are not sampled in this study, but they would undoubtedly be placed within the $x = 7$ clade. In the PACCAD Clade, the base number $x = 12$ occurs in several disparate taxa and is interpreted as the plesiomorphic state of this clade. Among the many other base numbers in the PACCAD Clade, there are a few unambiguous autapomorphic transformations (e.g., to $x = 9$ in *Molinia* and *Sporobolus*), but only one unambiguous synapomorphic transformation, to $x = 11$, in Aristidoideae.

Biochemistry

48 (7 steps, CI = 0.57, RI = 0.75). Carbon fixation pathways: All of the non-grass outgroups and all grasses outside the PACCAD Clade share the C_3 photosynthetic pathway. Taxa with all five C_4 subtypes, as well as additional C_3 taxa, occur within the PACCAD Clade. These are intermixed to such an extent that this character would be homoplasious even if all subtypes of C_4 carbon fixation had been scored as a single state. One unambiguous point of origin of C_4 carbon fixation is the transformation to NADP-ME decarboxylation (state 1) as a synapomorphy of Panicoideae (although recent studies on the phylogeny of Panicoideae show that even this is ambiguous (Giussani et al., in press)). The occurrence of NAD-ME decarboxylation (state 3) in *Panicum* is interpreted as a secondary transformation from NADP-ME. Outside of the Panicoideae and the Chloridoideae (discussed below), the occurrence of two different types of C_4 decarboxylating enzymes in taxa with and without mestome sheaths (states 2, 3, and 5, in *Aristida*, *Stipagrostis*, and *Eriachne*, respectively) requires three additional transformations, but various sequences of transformation among states can explain the variation observed in these three genera. Finally, the predominant occurrence of the NAD-ME type of decarboxylation in Chloridoideae, including *Centropodia* but not *Merxmuellera rangei* (which has C_3 photosynthesis), suggests a synapomorphic gain of this syndrome in the ancestor of Chloridoideae, followed by a return to C_3 photosynthesis in *Merxmuellera rangei*, but multiple gains of the NAD-ME subtype also are possible. Reversal from C_4 to C_3 also occurs in *Eragrostis walteri* (van den Borre, 1994). State 4, the NADP-ME *Arundinella*-type, occurs in *Arundinella*, some species of *Microstegium*, *Arthraxon*, and in some species of *Danthoniopsis*, which is here scored as polymorphic.

49 (1 step, CI = 1.0, RI = 1.0). PCK-type carbon fixation, p/a: This syndrome arises once, as an unambiguous transformation from the "normal" NAD-ME C_4 syndrome in a subclade of the Chloridoideae. *Zoysia* and *Spartina* exhibit PCK-type carbon fixation, and *Sporobolus* is polymorphic for presence/absence of this character.

Indel in Phytochrome B

50 (2 steps, CI = 0.50, RI = 0.92). 3-bp deletion in phytochrome, p/a: The deleted genotype (state 1) occurs in all sampled taxa of the PACCAD Clade, while the undeleted genotype (state 0) occurs in all other sampled grasses. *Joinvillea* has a 3-bp deletion and *Flagellaria* a 12-bp deletion in this region. *Sesleria* (not included in the present taxon sample) also has a 3-bp deletion (Mathews et al., 1995). The deletion occurs in a region of exon 1 characterized by extensive length and nucleotide variability, and it seems likely that grasses outside the PACCAD Clade have a synapomorphic insertion and that the deletion in *Sesleria* is apomorphic. Under this reconstruction, the deleted genotype in the PACCAD Clade would be synapomorphic, but the absence of data for *Micraira* precludes unambiguous optimization of the transformation, which is either a synapomorphy of the entire PACCAD Clade (including *Micraira*) or of the subclade that consists of all taxa except *Micraira*.

Chloroplast Genome Structure

51 (1 step, CI = 1.0, RI = 1.0). 6.4 kb inversion in the chloroplast genome, p/a: Absence of the inversion in *Flagellaria* and *Baloskion* (no information for *Elegia*), coupled with presence in *Joinvillea* and all grasses that have been sampled, allows unambiguous optimization of this character as a synapomorphy of *Joinvillea* plus Poaceae.

52 (1 step, CI = 1.0, RI = 1.0). *trn*T inversion in the chloroplast genome, p/a: Absence of this inversion in *Flagellaria* and *Joinvillea* (no information for either genus of Restionaceae in the present study), coupled with presence in all grasses that have been sampled, allows unambiguous optimization of this character as a synapomorphy of Poaceae. Although this character has not been scored for *Elegia* or *Baloskion*, it has been scored in another genus of Restionaceae, *Chondropetalum* (Doyle et al., 1992), and absence of the inversion in that genus is consistent with the interpretation that this inversion is a synapomorphy of Poaceae.

53 (3 steps, CI = 0.33, RI = 0.71). 15 bp *ndh*F insertion, p/a: This insertion was previously reported as present in all sampled grasses except Anomochlooideae, Pharoideae, and Oryzeae (though present in Ehrharteae), and absent outside the Poaceae (Clark et al., 1995). New sequences reported here confirm this distribution except for the presence of the insertion in *Elegia*. Thus, the insertion arises independently as an autapomorphy of *Elegia* and as a synapomorphy of the Bistigmatic Clade (all grasses except Anomochlooideae and Pharoideae); it is secondarily lost (i.e., deleted) in the ancestor of (or within) the Oryzeae. Examination of this character in other Restionaceae and related families such as Anarthriaceae, Ecdeiocoleaeae, and Centrolepidaceae is warranted.

PHYLOGENY, RADIATION, AND TRANSOCEANIC DISPERSAL OF NEW ZEALAND ALPINE BUTTERCUPS: MOLECULAR EVIDENCE UNDER SPLIT DECOMPOSITION[1]

Peter J. Lockhart[2], *Patricia A. McLenachan*[2], *David Havell*[3], *David Glenny*[4], *Daniel Huson*[5], *and Uwe Jensen*[6]

Abstract

The "Alpine Ranunculi of New Zealand" are a monophyletic group of species distributed between the New Zealand alps, Australian alps, and the subantarctic Campbell and Auckland Islands. For this group we determined and analyzed sequences for the nuclear ITS and chloroplast J_{5A} regions. This latter region was identified from an amplified fragment length polymorphism (AFLP) gel as being potentially phylogenetically informative. We have used quartet puzzling to represent the overall phylogenetic structure of these sequence data and split decomposition to investigate more closely the phylogenetic information among sequences from two lineages within the alpine radiation. We describe molecular evidence that shows that diversification of alpine Ranunculi has accompanied the onset of Pliocene mountain building in New Zealand, and that during range expansion of species, regional speciation into novel alpine habitats has occurred with parallel evolution of morphologies between more distantly related species. Our analyses also show that, during diversification and range expansion, the New Zealand alps acted as a center for long-distance dispersal to Australia and the New Zealand subantarctic islands.

Key words: alpine radiation, dispersal, phylogeny, quartet puzzling, *Ranunculus*, split decomposition.

One of the most complete and detailed studies of the radiation of an alpine plant group is on the "Alpine Ranunculi of New Zealand" by Fisher (1965). In Fisher's work, 14 species were recognized in this group, i.e., *R. buchananii, R. crithmifolius, R. enysii, R. godleyanus, R. gracilipes, R. grahamii, R. haastii, R. insignis, R. lyallii, R. nivicola, R. pachyrrhizus, R. pinguis, R. sericophyllus,* and *R. verticillatus.* Fisher (1965) also described two subspecies each for *R. crithmifolius* and *R. haastii.* Subsequently two further species were also described in New Zealand. These were *R. scrithalis* P. J. Garnock-Jones and *R. viridis* H. D. Wilson & P. J. Garnock-Jones (see Webb et al., 1988). These 18 taxa occur in a wide range of alpine habitats in the New Zealand archipelago and include *R. pinguis,* which is found on the subantarctic Campbell and Auckland Islands. In addition, *R. anemoneus* and *R. gunnianus,* which occur in the Australian alps, have also been suspected of being part of this group (Fisher, 1965). All 20 taxa have been placed into section *Epirotes* (Prantl) L. Benson (see Fisher, 1965; Webb et al., 1988). Tamura (1995) transferred them into section *Pseudadonis* F. Muell. They are the subject of our present study (the ingroup taxa in Table 1). Other indigenous (and more or less lowland distributed) *Ranunculus* species also occur in New Zealand. They belong to section *Epirotes* and to section *Chrysanthe* (Spach) L. Benson, which has a worldwide distribution. The phylogenetic relationships within these groups are not investigated in our present study.

The alpine Ranunculi of New Zealand are particularly noted for their diverse and characteristic morphologies. Leaf shapes range from entire (*R. insignis*) to pinnately compound (*R. gracilipes*). Spe-

[1] This work was supported by grants from the New Zealand Marsden Fund, The Alexander von Humboldt Foundation, FRG/NZ ISAT programme, and Massey University. Many thanks to Tristan Armstrong, Peter Henman, Heidrun Lehmann, Vivian Nicholls, Alastair Robertson, Mike Steel, Brian and Chris Rance, the New Zealand Department of Conservation, and Te Runanga O Ngai Tahu for help with obtaining plant material. Thanks to Richard Winkworth for helpful comments on the manuscript and to Kim McBreen and Lorraine Berry (Museq) for help with sequencing.

[2] Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand.
[3] Universal College of Learning, Palmerston North, New Zealand.
[4] Landcare Research, Lincoln, New Zealand.
[5] Program in Applied and Computational Mathematics, Princeton University, U.S.A.
[6] Fliederweg 8, D-24211 Preetz, Germany.

Table 1. *Ranunculus* species used for research.

Ingroup
Ranunculus subg. *Ranunculus* Peterm. 1846.
Sect. *Pseudadonis* F. Muell. 1860.
Syn.: Sect. *Epirotes* (Prantl) L. Benson 1936. 26–33, 169–176.
Alpine group Fisher, Alp. Ran. New Zealand 1965.
R. anemoneus F. Muell., Trans. Philos. Soc. Victoria 1855. 1: 97.
R. buchananii Hook. f., Handbk. New Zealand Fl. 1864. 5.
R. crithmifolius Hook. f., Handbk. New Zealand Fl. 1864. 6.
subsp. *crithmifolius* Fisher, Alp. Ran. New Zealand 1965.
subsp. *paucifolius* (T. Kirk) Fisher, Alp. Ran. New Zealand 1965.
R. enysii T. Kirk, Trans. New Zealand Inst. 1880. 12: 394.
R. godleyanus Hook. f., Handbk. New Zealand Fl. 1867. 723.
R. gracilipes Hook. f., Handb. New Zealand Fl. 1864. 8.
R. grahamii Petrie, Trans. New Zealand Inst. 1914. 46: 32.
R. gunnianus Hook., Hook. J. Bot. 1834. 1: 244.
R. haastii Hook. f., Handbk. New Zealand Fl. 1864. 6.
subsp. *haastii* Fisher, Alp. Ran. New Zealand 1965.
subsp. *piliferus* Fisher, Alp. Ran. New Zealand 1965.
R. insignis Hook. f., Fl. New Zealand 1852. 1: 8.
R. lyallii Hook. f., Handbk. New Zealand Fl. 1864. 4.
R. nivicola Hook., Ic. 1844. 571–572.
R. pachyrrhizus Hook. f., Handbk. New Zealand Fl. 1864. 8.
R. pinguis Hook. f., Fl. Antarct. 1844. 3.
R. scrithalis P. J. Garnock-Jones, New Zealand J. Bot. 1987. 25: 126.
R. sericophyllus Hook. f., Handbk. New Zealand Fl. 1864. 6.
R. verticillatus T. Kirk, Fl. New Zealand 1899. 13.
R. viridis H. D. Wilson & P. J. Garnock-Jones, New Zealand J. Bot. 1983. 21: 342.
Outgroups
Ranunculus subg. *Ranunculus* Peterm. 1846.
Sect. *Pseudadonis* F. Muell. 1860.
Syn.: Sect. *Epirotes* (Prantl) Benson 1936. 26–33, 169–176.
Lowland group Fisher, Alp. Ran. New Zealand 1965.
R. acaulis Banks & Soland. ex DC., Re. Veg. Syst. Nat. 1817. 1: 270.
Sect. *Hecatonia* (Lour.) DC. 1824.
R. sceleratus L., Sp. Pl. 1753. 551.
Sect. *Chrysanthe* (Spach) L. Benson 1936.
R. recens T. Kirk, Fl. New Zealand 1899. 13.
Ranunculus subg. *Batrachium* (DC.) Peterm. 1816.
R. circinatus Sibth., Fl. Oxon. 1794. 175.

cies are found in habitats ranging from those above the permanent snow line, on stone or silt screes, to grassland, swamps, or sheltered situations. Breeding studies carried out on most, but not all species have suggested the existence of two reproductively incompatible groups of species (Fisher, 1965). Following the onset of mountain building in the Pliocene (Cooper & Millener, 1993; Batt et al., 2000), diversification in different breeding groups is thought to have led to the evolution of similar morphologies in species occupying similar alpine niches (Fisher, 1965). This is considered to have been a consequence of alpine geological boundaries, which limited seed dispersal and restricted species from occupying suitable but geographically disjunct sites in different parts of New Zealand (Fisher, 1965). Origins of the alpine Ranunculi of New Zealand are unclear, and their distribution between Australia, New Zealand, and their subantarctic islands has given rise to different hypotheses of evolutionary relationships and long-distance dispersal (Fisher, 1965; Raven, 1973; Wardle, 1978).

Molecular systematics provides an opportunity to revisit hypotheses raised by Fisher's earlier botanical and breeding studies on the alpine Ranunculi of New Zealand. Our work reported here describes molecular data and analyses that allow tests to be made between patterns of radiation, between hypotheses seeking to explain the geographic distribution of the group and whether speciation occurred following the onset of Pliocene mountain building in New Zealand. An amplified fragment length polymorphism (AFLP) method was used previously to find a highly variable DNA sequencing marker at the chloroplast J_{SA} region (the junction of the inverted repeat IR_A and small single-copy region SSC). The phylogenetic information from this marker has been compared in the present study

Table 2. General information about the voucher origin. N.Z.: New Zealand; S.Isl.: South Island; N.Isl: North Island; Mts.: Mountains; alp.: alpine; *R.*: *Ranunculus*.

Taxa	General distribution and habitat	Voucher, locality, collector	GenBank accessions ITS	GenBank accessions JSA
R. gunnianus	Australian Alps, wet tussock and grassland	MPN24587, Mt. Kosciusko, *T. Armstrong*	AF323298	AF323348
R. anemoneus	Australian Alps: Mt. Kosiusko, snow line fringe	MPN24588, Mt. Kosciusko, *T. Armstrong*	AF323273	AF323323
R. pinguis	Subantarctic Campbell and Auckland Islands, windswept sodden grassland and rocky moors	MPN24590, Campbell Island, *V. Nicholls*	AF323299	AF323349
		MPN24591, Auckland Island, *V. Nicholls*	AF323300	AF323350
R. sericophyllus	N.Z.: S.Isl., wet western southern alps, snowline fringe	MPN24595, Mt. Tutoko, *M. Steel*	AF323288	AF323338
		CHR529050, Lake Wapiti, *D. Glenny*	AF323289	AF323339
		CHR530524, Gertrude Valley: Black Lake, *D. Glenny*	AF323290	AF323340
		MPN24596, Mt. Cook, *M. Steel*	AF323291	AF323341
		MPN24597, Mt. Franklin, *M. Steel*	AF323292	AF323342
		MPN24667, Temple Basin, *M. Steel*	AF323293	AF323343
		MPN24668, Mt. Memphis, *D. Havell*	AF323294	AF323344
R. pachyrrhizus	N.Z.: S.Isl., drier eastern southern alps	MPN24598, Pisa Range, *D. Havell*	AF323295	AF323345
		MPN24599, Old Man Range, *A. Robertson*	AF323296	AF323346
R. viridis	N.Z.: Stewart Isl., granite ledges	No voucher (vulnerable/endangered), Mt. Allen, *D. Havell*	AF323297	AF323347
R. scrithalis	N.Z.: S.Isl., Eyre mountains, clay scree	MPN24600, Hummock Peak, *P. Lockhart*	AF323305	AF323355
R. lyallii	N.Z.: S.Isl., high rainfall areas on well-drained soils, gullies, shrubland, grassland	MPN24601, Mt. Tutoko, *M. Steel*	AF323283	AF323333
		MPN24602, Mt. Franklin, *M. Steel*	AF323274	AF323324
		MPN24603, Mt. Cook, *M. Steel*	AF323277	AF323327
		CHR 306134A, Mt. George, *D. Given*	AF323278	AF323328
		CHR110835, Hump Ridge, *E. J. Godley*	AF323282	AF323332
		MPN24669, Temple Basin, *M. Steel*	AF323275	AF323325
		MPN24670, Franz Josef, *D. Havell*	AF323276	AF323326
R. buchananii	N.Z.: S.Isl., southern alps, snowline fringe, scree	CHR509922, Garvie Mts.: above Skeleton Lake, *D. Glenny*	AF323280	AF323330
		CHR529051, Lake Wapiti, *D. Glenny*	AF323281	AF323331
		MPN24761, Takatimu Range: Clare Peak, *R. Havell*	AF323279	AF323329
R. haastii subsp. *haastii*	N.Z.: S.Isl., eastern alp. distribution, stone debris	CHR518452, Mt. Hutt, *P. Heehnan*	AF323284	AF323334
		CHR532284, Amuri Ski field, *D. Glenny*	AF323285	AF323335

Table 2. Continued.

Taxa	General distribution and habitat	Voucher, locality, collector	GenBank accessions ITS	GenBank accessions JSA
R. haastii subsp. *piliferus*	N.Z.: S.Isl., eastern alp. distribution, stone debris species	CHR509769, Eyre Mts., Helen Peaks, *D. Glenny*	AF323301	AF323351
		MPN24604, Eyre Mts.: Hummock Peak, *P. Lockhart*	AF323302	AF323352
R. grahamii	N.Z.: S.Isl., Mt. Cook region, rocky crevices and ledges above the permanent snowline	CHR217997, Ben Ohau Range: Twins Basin, *A. C. Archer*	AF323286	AF323336
		MPN24672, Malte Brun: Aiguilles Rouge, *M. Steel*	AF323287	AF323337
R. insignis	N.Z.: N.Isl. and central–northern S.Isl., sheltered situations	MPN24605, S.Isl.: Foggy Peak, *D. Glenny*	AF323306	AF323356
		MPN24606, N.Isl.: Volcanic Plateau, *D. Havell*	AF323307	AF323357
R. verticillatus	N.Z.: southern N.Isl. and central– northern S.Isl., grasslands	MPN24607, N.Isl.: Mt Holdsworth, *D. Havell*	AF323303	AF323353
		CHR530493, S.Isl.: Cobb Valley: Mt. Mytton, *D. Glenny*	AF323304	AF323354
R. nivicola	N.Z.: N.Isl.: Volcanic Plateau to Mt. Hikurangi, generally higher altitudes than *R. insignis* and *R. verticillatus*, grassland and stone debris	MPN24608, Volcanic Plateau, *P. Lockhart*	AF323308	AF323358
R. godleyanus	N.Z.: central S.Isl., rocky places at the snowline fringe	CHR499398, Havelock River: Eric Sream, *M. Harding*	AF323309	AF323359
		MPN24609, Malte Brun, *M. Steel*	AF323310	AF323360
R. crithmifolius subsp. *paucifolius*	N.Z.: S.Isl., Castle Hill only, limestone fine scree	No voucher (endangered), Castle Hill, *P. Lockhart*	AF323312	AF323362
R. crithmifolius subsp. *crithmifolius*	N.Z.: S.Isl., stony debris	CHR509774, Eyre Mts: Shepherd Saddle, *D. Glenny*	AF323311	AF323361
		MPN24673, Eyre Mts.: Big Jungle Creek, *B. Rance*	AF323313	AF323363
R. enysii	N.Z.: S.Isl., eastern alp. distribution, sheltered situations	CHR509805, Clarence River: Island Pass, *D. Glenny*	AF323316	AF323366
		MPN24610, Nevis Valley, *B. Rance*	AF323317	AF323367
		MPN24611, Umbrella Mts., *B. Rance*	AF323318	AF323368
R. gracilipes	N.Z.: S.Isl., damp and tussock grassland, scrub and sheltered rocky places	CHR529048, Remarkables: Lake Alta, *D. Glenny*	AF323314	AF323364
		MPN24612, Eyre Mts.: Symmetry Peaks, *P. Lockhart*	AF323315	AF323365
R. acaulis	N.Z., subantarctic Islands, Australia, Southern Chile, coastal habitats	MPN24592, Stewart Isl.: Masons Bay, *D. Havell*	AF323319	-
R. sceleratus	Europe, North America, spread worldwide	MPN24589, Germany: Oberpfalz, *H. Iehmann*	AF323322	-
R. circinatus	Eurasia, aquatic habitats	MPN24671, Germany: Franken, *H. Iehmann*	AF323321	-
R. recens	N.Z., lowland coastal, local populations	No voucher (endangered), Stewart Isl.: Masons Bay, *D. Havell*	AF323320	-

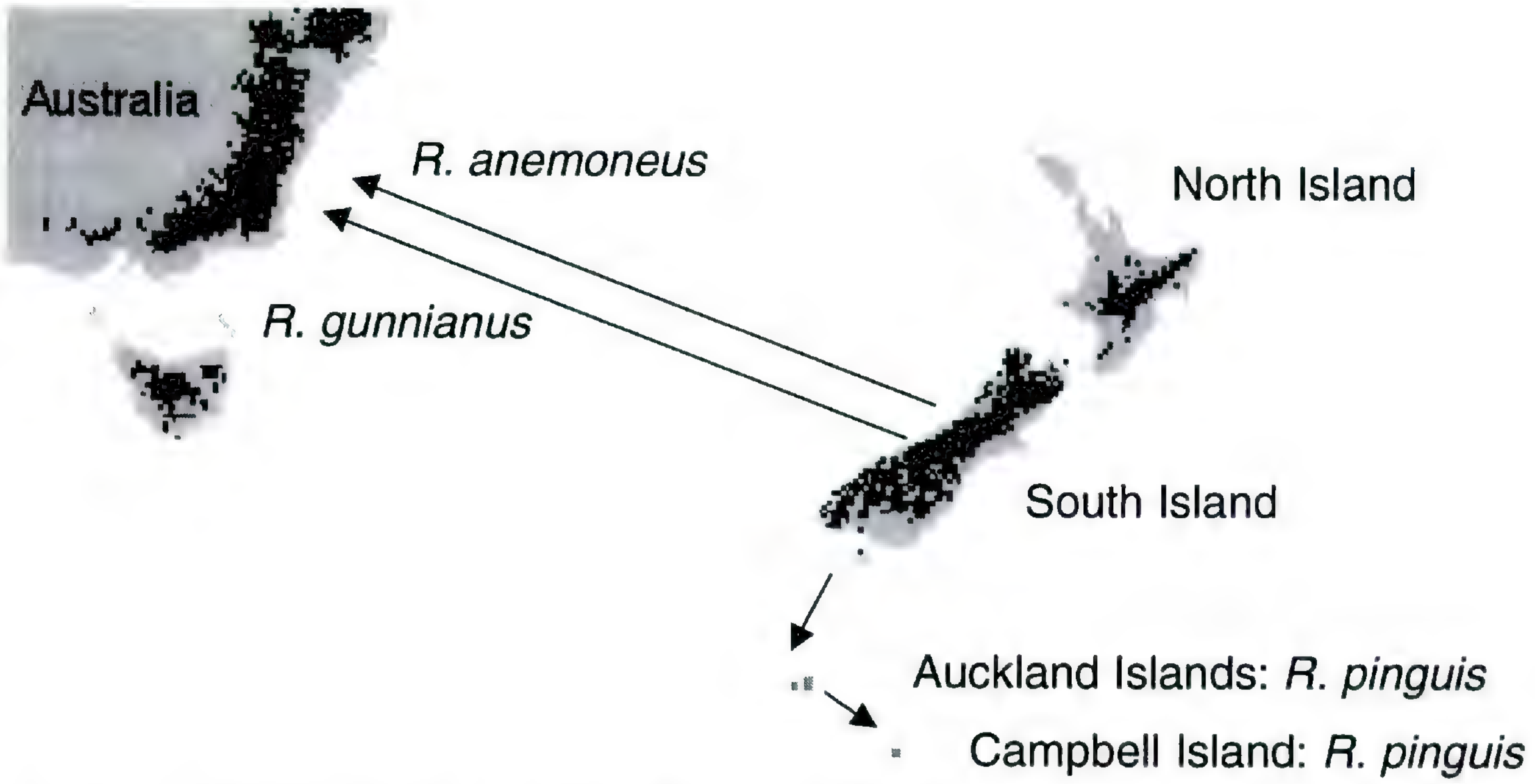


Figure 1. Geographic context of the alpine Ranunculi of New Zealand. The species labeled are those that have arisen following transoceanic dispersal from New Zealand in the directions indicated by arrows.

with that of the commonly used nuclear ITS marker (Johnson & Soltis, 1995). We have studied these data under quartet puzzling (Strimmer & von Haeseler, 1996) and split decomposition (Bandelt & Dress, 1992; Huson, 1998). The first method was used to obtain a rooted phylogenetic and graphic representation of the data. We used split decomposition to investigate more closely the phylogenetic relationships within two monophyletic groups from the New Zealand alpine radiation.

Materials and Methods

Plant material, distributions, and geography

Table 1 provides the authorities and references for the ingroup and outgroup species studied in the present work. *Ranunculus acaulis*, *R. sceleratus*, and *R. circinatus* were selected as outgroups based on the results of an earlier molecular study (Johansson, 1998). Table 2 gives details of the habitats, collection sites, names of collectors, herbarium vouchers, and GENBANK accession numbers. Vouchers for accessions are held at the Massey University herbarium (MPN) and the Landcare herbarium, Lincoln (CHR). The geographic context of our study is shown in Figure 1. The New Zealand distributions of alpine *Ranunculus* species are shown in Figure 2.

Identifying hypervariable regions in plant genomes

Experimental protocols that examine restriction endonuclease site differences between plant genomes can provide a helpful means to detect rapidly evolving plant genome regions. In a preliminary study we used the AFLP method of Vos et al. (1995) with AT-rich selective primers in an attempt to identify rapidly evolving length mutations (Golenberg et al., 1993) and restriction site differences in the chloroplast and mitochondrial DNAs of New Zealand alpine Ranunculi (Lockhart & McLenachan, 1997). Length mutations can occur due to the presence of small monomeric and direct repetitive sequences (Powell et al., 1995; Golenberg et al., 1993) and appear in genome regions that show elevated substitution rates. They have been found in chloroplast genomes at sites of intramolecular recombination such as in the single copy regions (at tRNA and *acc*D loci: Maier et al., 1995; Yasui & Ohnishi, 1998), and at the junctions of inverted repeat/single copy regions. These include those at the J_{LA} region: *mat*K-*psb*A (Powell et al., 1995; Johnson & Soltis, 1995), J_{LB} region: the *rpl*16 intron (Kelchner & Clark, 1997), and the J_{SB} region: *ndh*F (Olmstead & Sweere, 1994). Regions with direct repeats and elevated substitution rates have also been reported in DNAs of mitochondrial origin (e.g., Luo & Boutry, 1995).

One polymorphic region identified in our *Ranunculus* AFLP profiles was the chloroplast J_{SA} region (Lockhart & McLenachan, 1997). This locus appears to be previously unstudied in molecular systematic sequencing studies. Based on DNA sequence determined for a cloned AFLP profile band, we designed PCR primers (5′ATTATYAATGAAGGYAATACWATATATTTTC 3′ and 5′CAAATTCCAATGAC-

CAAATAGTTCG 3′) with which, using a standard PCR thermocycling profile (94°C 2 min., followed by 35 cycles: 94°C 30 secs., 50°C 1 min., 72°C 1 min., 72°C 5 min.), we amplified a portion of the J_{SA} region in species of New Zealand alpine Ranunculi. Due to the presence of direct repeats in the sequences of some taxa, this fragment ranged in size from 493 to 511 bp. We also amplified and sequenced 598–603 bp of the nuclear ITS (ITS 1, 5.8S rDNA, ITS 2) region using standard primers (from Baldwin et al., 1995). For both J_{SA} and ITS regions, sequencing was done for multiple accessions of most New Zealand alpine species. Characterization of PCR products was made for both DNA strands using an ABI377 sequence protocol. Aligned sequences have been submitted to GENBANK and are available with the accession numbers AF323323–AF323368; AF323273–AF323308. More recently we have also used AFLP to find molecular markers for polymorphic genome regions in *Nothofagus* (Nothofagaceae), *Myrsine* (Myrsinaceae), *Rhopalostylis* (Palmae), *Myosotis* (Boraginaceae), and *Phormium* (Phormiaceae) (McLenachan et al., 2000).

Phylogenetic Reconstruction and Plant Speciation

quartet puzzling

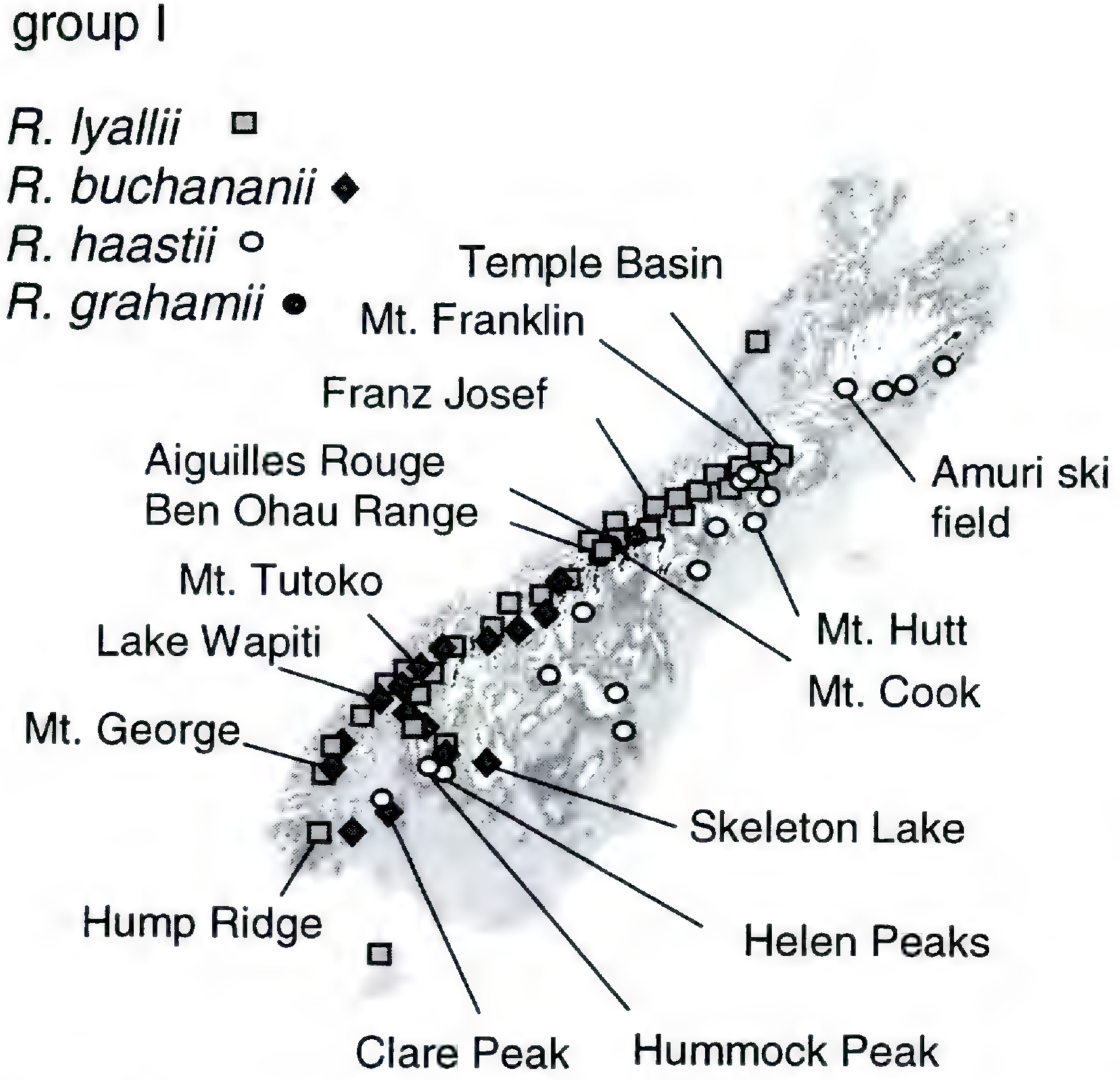
Quartet puzzling (QP, Strimmer & von Haeseler, 1996) was used to represent the phylogenetic structure of the main groups and lineages within the New Zealand alpine radiation. Evolutionary trees were reconstructed using the implementation of QP in PAUP*4.0b3a (Swofford, 1998). With the ITS data, outgroups were used to indicate the direction of evolution within the graph. Support for quartets was evaluated under minimum evolution criteria (Swofford et al., 1996) using observed distances that included insertion and deletion characters. With the ITS data, heteroplasmic sites were removed before tree building. QP is a heuristic tree building procedure that works efficiently (Penny et al., 1996) with a large number of taxa, to give a bifurcating tree onto which edge (branch) lengths can then be estimated. In QP phylogenetic trees, the support values assigned to groupings of taxa are usually interpreted as similar to bootstrap values (Strimmer & von Haeseler, 1996). However, they can differ considerably from bootstrap values in the case of recently diverged sequences, such as in the study of Late Tertiary–Quaternary plant radiations. Unlike bootstrap values, QP values are usually high when sequence data show few informative sites but relatively few incompatibilities. The problem of interpreting low bootstrap values in molecular studies of recently evolved taxa has been noted elsewhere (Knox & Palmer, 1995; Bandelt et al., 1995). QP values provided us with an indication in our molecular data of the degree of compatibility for phylogenetic groupings.

a problem with bifurcating evolutionary tree models

Phylogenetic analysis of DNA sequences from Late Tertiary–Quaternary plant radiations can be complicated when molecular markers showing suitable levels of variation exist at multiple loci in plant genomes (e.g., Buckler et al., 1997; Shan et al., 1999; Linder et al., 2000; McLenachan et al., 2000). When these sequences show differences from each other, bifurcating evolutionary tree models (such as commonly implemented under parsimony, neighbor joining, and maximum likelihood selection criteria) usually provide a poor representation of the phylogenetic complexity of the sequence information (e.g., Sang et al., 1994; Koch & Al-Shehbaz, 2000). These more commonly used approaches to tree building also provide poor representation when extant taxa are characterized by sequences that have not diverged from those of ancestors. In this case, commonly used methods tend to force taxa away from internal points in the tree and onto bifurcating tips of the reconstructed tree.

Split Decomposition

There seems to exist no simple solution to the problem of fully representing the phylogenetic information in sequence data and at the same time maintaining a low order of computational complexity in analysis. However, split decomposition (Bandelt & Dress, 1992; Huson, 1998) is one efficient approach that provides a means to investigate and represent more complex substitution patterns in an alignment of sequences (e.g., Lockhart et al., 1995). Presently implemented under parsimony or distance criteria (Huson, 1998), it allows investigation of whether the data structure is or is not tree-like and bifurcating. If sequences show evidence of recombination this will be represented in the form of reticulate graphs. In the present study we used the implemention of split decomposition in SplitsTree 3.1 (Huson, 1998) to examine the relationships between taxa belonging to two lineages within the New Zealand alpine radiation. The relationships within a third New Zealand alpine lineage have been studied elsewhere (Huber et al., 2001) using median and pruned median networks—which are methods also seeking to more fully represent the



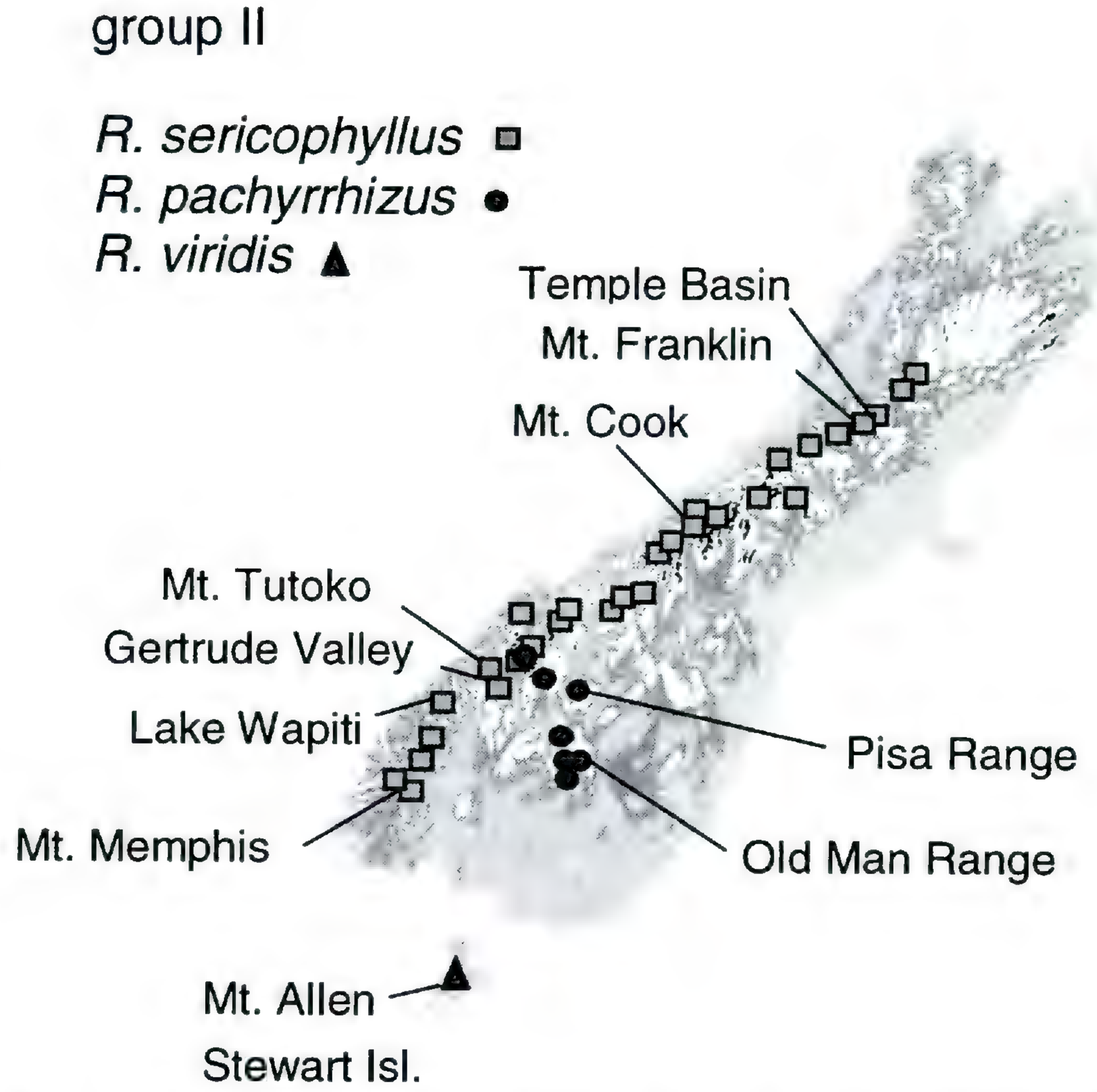


Figure 2. Distributions of taxa from different phylogenetic groupings (I–IV) in the North, South, and Stewart Islands. Sites of known collections are indicated by symbols. These locations are reported in Fisher (1965), Webb et al. (1988), and identified in our own collections. The sites at which sample collections were made in the present study are indicated by names.

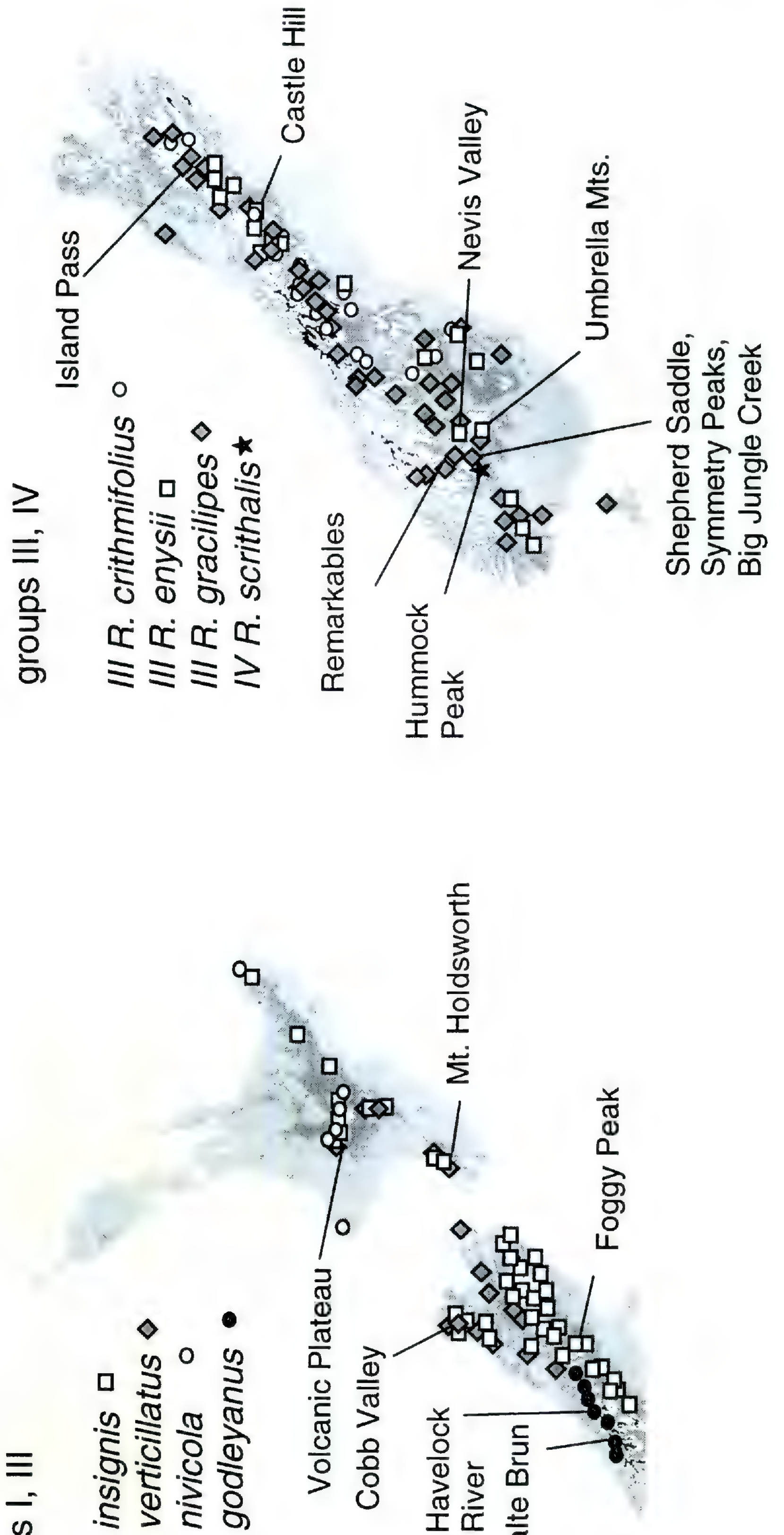


Figure 2. Continued.

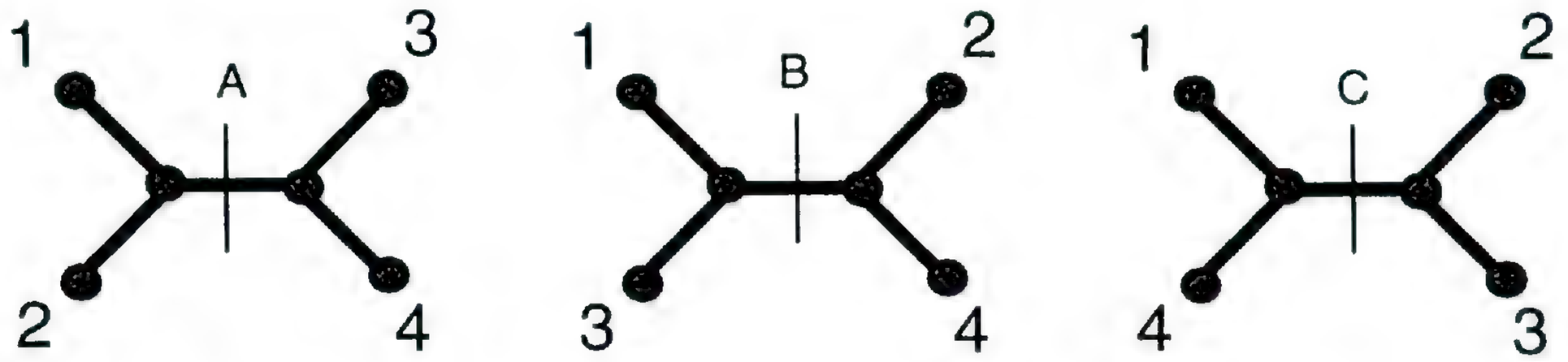


Figure 3. The splits A, B, and C uniquely identify the three possible quartets for the taxa 1, 2, 3, 4.

phylogenetic complexity of sequence data. We did not include insertions and deletions in our splits graphs, which were calculated from observed distances. These indels were all compatible with splits in the splits graphs shown and have been indicated in the figure legends. Since split decomposition has rarely been used by plant systematists (but see Bandelt, 1995; Koch & Al-Shehbaz, 2000), we provide a simple description of the approach. The procedure involves three distinct steps:

(A) FINDING WEAKLY COMPATIBLE SPLITS

First, all possible combinations of four taxa from a data set (perhaps containing many taxa) are examined. For each combination of four taxa, support is evaluated for each of the internal "splits" that uniquely identifies the possible bifurcating trees (for every combination of four taxa, three bifurcating trees are possible: Fig. 3).

Parsimony, distance, or maximum likelihood (or other) criteria can be used in deciding which two of the three bifurcating trees are most strongly supported by the data. Here, we use a distance calculation to obtain measures of support (isolation index values). For split A (Tree 1) the isolation index $= 0.5 \times (d_{14} + d_{23} - d_{12} - d_{34}) = 0.5 \times (d_{24} + d_{13} - d_{12} - d_{34})$; for tree split B (Tree 2) the isolation index $= 0.5 \times (d_{14} + d_{23} - d_{13} - d_{24})$; and for tree split C (Tree 3) the isolation index $= 0.5 \times (d_{13} + d_{24} - d_{14} - d_{23})$ where d is the path length between the pairs of taxa (e.g., perhaps the observed number of differences between the taxa). The two splits that have the highest isolation index score for each combination of four taxa are considered to be weakly compatible, and they are used to next obtain a split system that describes the relationship between *all* taxa in the original data set.

(B) OBTAINING A SPLIT SYSTEM

The weakly compatible splits from the quartet study define the presence of the internal splits that will also occur in the split system for all taxa in the data set. Since some of the quartets not excluded in the first step of the quartet study identify the *same* split between more than four taxa, a decision needs to be made as to which isolation index value will be used. For example, consider one particular split in a split system (as shown in Fig. 4). This split (A) separates taxa 1 and 2 from taxa 3, 4, and 5. The split is equivalently identified by the quartet splits: taxa 1 and 2 split from taxa 3 and 4; taxa 1 and 2 split from taxa 3 and 5; taxa 1 and 2 split from taxa 4 and 5; etc. In its standard implementation split decomposition chooses the *smallest* isolation index value from all the quartet splits that are compatible with that split in the split

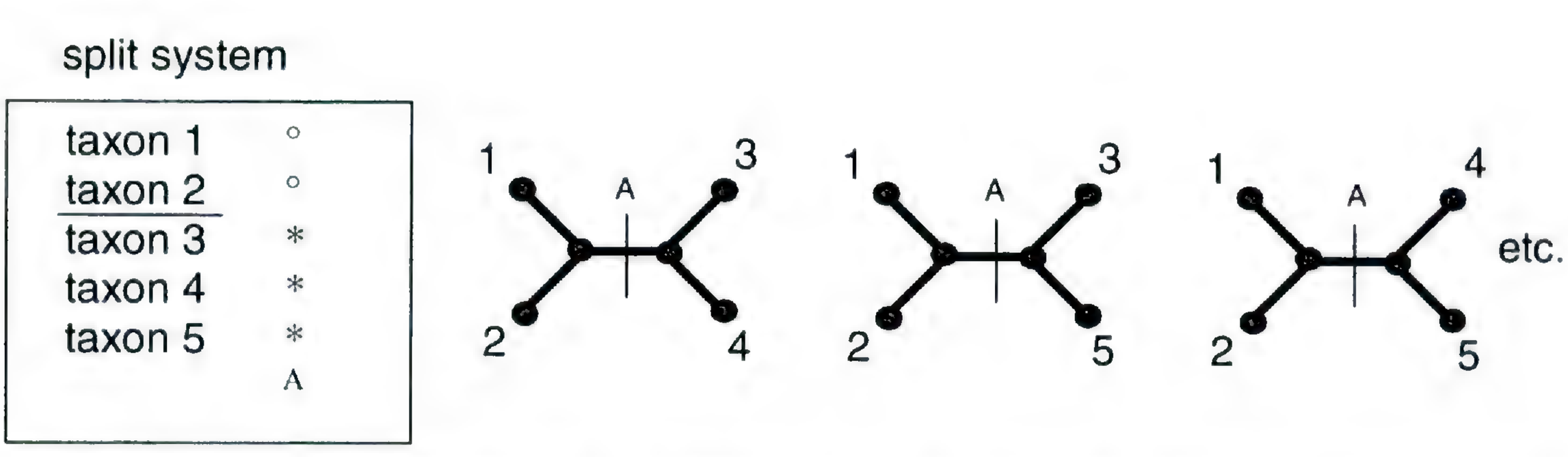


Figure 4. In the split system shown, split A separates taxon 1 and 2 from taxa 3, 4, and 5. Symbols (○, .) have been used to identify the taxa on either side of split A. This symbol representation is also used to identify the different splits in the split system shown in Figure 5.

system. The rationale for choosing the smallest value is that the method will be conservative in deciding whether there is really support for such a split in the split system. An alternative or less conservative approach is to average across values for compatible quartet splits (Moulton et al., 1997; Huson, 1998).

The split system must also contain the splits that correspond to the external edges or branches in the final tree or network. Support for these is calculated using all combinations of three taxa. If such taxa are identified as i, j, k then the isolation index for the split (edge) leading to $i = 0.5 \times (d_{ij} + d_{ik} - d_{jk})$. Split decomposition chooses the smallest value for the length of that split.

(c) Building a Splits Graph

Once the splits present in a split system have been identified and their values assigned, a graph is constructed from the split system by an algorithm that splits taxa from each other (e.g., see Fig. 5).

If the split system contains no incompatibilities (as in split system i, or in the more resolved split system ii), a tree-like structure will result. If there are incompatibilities a network (boxes) will appear (split system iii). In this graph, the edges (internodes) have lengths that correspond to the isolation index values of the splits in the split system. In some cases, these lengths will be an approximation of those in the distance matrix, in which case a lower fit statistic is associated with the graph (the fit statistic = sum of all the paths in the graph divided by the sum of all paths in the pairwise distance matrix). In splits graphs, genotypes are not forced onto the tips of bifurcating trees. Thus, splits graphs provide more informative graphic representations when sequences from the accessions examined are identical to those in ancestors. Splits graphs are also advantageous in that they highlight the strongest incompatible signals in data that indicate contradictory evidence for cladistic relationships.

A limitation with the current implementation of split decomposition is the underestimation of internal edge lengths when the number of taxa compared is large and when the divergence between some taxa is great. In the present study we used split decomposition to study very closely related taxa. In our example, the split decomposition fit statistic was always very high, indicating that our splits graphs well represented the information contained in the sequences studied.

Molecular Clock Analyses

We used the method of Steel et al. (1996) as implemented in Splitstree 3.1 (Huson, 1998) to examine the molecular clock-like properties of ITS1 and ITS2 sequences for the two alpine *Ranunculus* breeding groups identified by Fisher (1965). This relative rates test compares the sequence differences between three taxa and examines whether the path lengths across the internal node that separates two of the taxa from the third are statistically equal. In the present study this investigation was made for all combinations of three taxa in our data matrix. To estimate the genetic distance (and subsequently time of divergence) between the two groups we also used a method suggested by Steel et al. (1996) implemented in Splitstree 3.1 (Huson, 1998). This procedure makes use of all available sequence data, within two groups, to help reduce the variance on the estimate of divergence between those two groups. Our estimate for the number of substitutions per site per million years was made with a Jukes-Cantor substitution model (Swofford et al., 1996) and compared against calibrated divergences of ITS1 and ITS2 sequences from an earlier study on *Dendroseris* (Sang et al., 1994). This comparison allowed us to obtain a tentative estimate for the divergence time of the two breeding groups.

Results

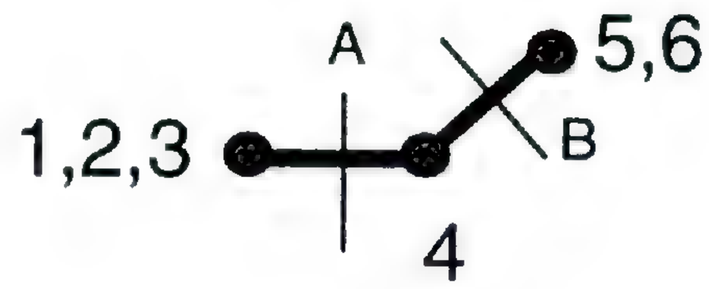
Four Alpine Groups

Quartet puzzle phylogenetic trees reconstructed from our ITS (ingroup and outgroup taxa) and J_{SA} (ingroup taxa) sequences are shown in Figure 6 and Figure 7, respectively. These results indicate that the alpine Ranunculi of New Zealand (species from within sect. *Pseudadonis*) comprise four distinct phylogenetic groups. *Group I* includes alpine species occupying (i) habitats at the snowline fringe: *R. anemoneus* (Australian alps) and *R. buchananii* (New Zealand Southern alps), (ii) wet well-drained habitats: *R. lyallii*, (iii) stony screes: *R. haastii* subsp. *haastii* and *R. haastii* subsp. *piliferus* (New Zealand Southern alps), (iv) habitats above the permanent snowline: *R. grahamii*, and (v) poorly drained alpine shrub and tussock land: *R. verticillatus* (but note that the chloroplast data suggest that *R. verticillatus* is distinct from this group). *Group II* comprises alpine and subantarctic species: the Australian alpine species *R. gunnianus*, the New Zealand snowline fringe species *R. sericophyllus* and *R. pachyrrhizus*, *R. viridis* from Mt. Allen on Stewart Island, and the subantarctic *R. pinguis* from Campbell and Auckland Islands. *Group III* contains

split system i

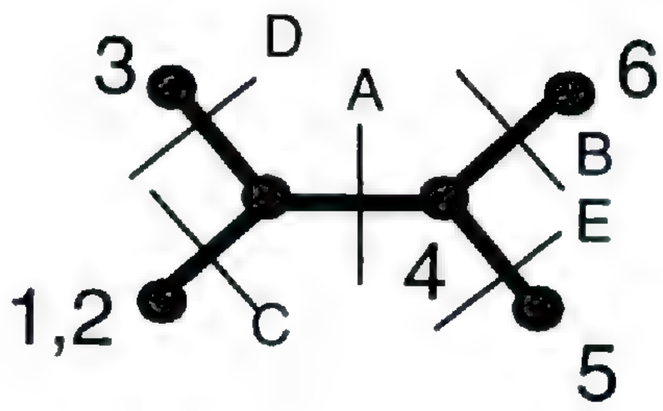
taxon 1	oo
taxon 2	oo
taxon 3	oo
taxon 4	*o
taxon 5	**
taxon 6	**
	AB

splits graphs



split system ii

taxon 1	ooo**
taxon 2	ooo**
taxon 3	oo*o*
taxon 4	*o***
taxon 5	*o**o
taxon 6	*****
	ABCDE



split system iii

taxon 1	ooo**o
taxon 2	ooo**o
taxon 3	oo*o**
taxon 4	*o***o
taxon 5	*o**oo
taxon 6	******
	ABCDEF

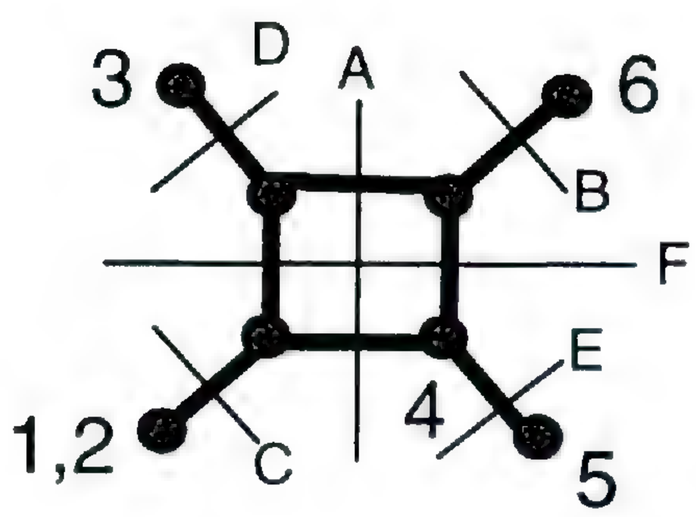


Figure 5. Examples of split systems and splits graphs. The splits (but not their lengths) in the split systems on the left have been used to construct the figures on the right.

alpine species having only a New Zealand distribution. These include both subspecies of the restricted scree specialist *R. crithmifolius*, the snowline fringe species *R. godleyanus*, and species occupying lower altitude sheltered situations: *R. insignis*, *R. enysii*, and *R. gracilipes*. *Group IV* is represented by a single alpine species, the scree specialist *R. scrithalis* from the Eyre mountains, South Island, New Zealand. The groupings in our trees are comparable with Fisher's (1965) two main phylogenetic groups, i.e., our groups I and II are Fisher's *many petals, silky hair* group, while group III

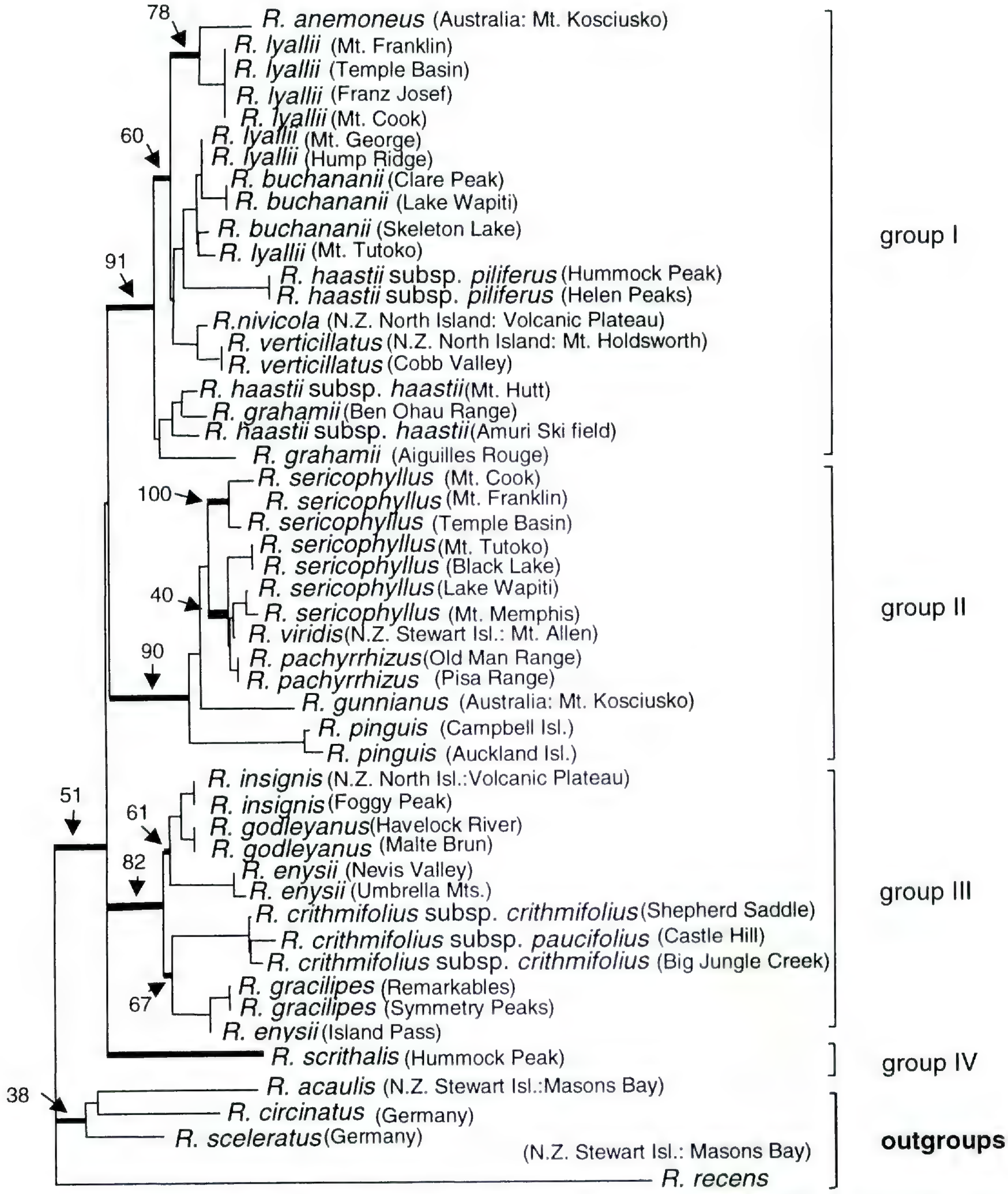


Figure 6. Quartet Puzzle tree showing branch lengths for ingroup and outgroup nuclear ITS sequences. Splits identifying groups I–IV are indicated by bold lines. The Quartet Puzzle support values for these and other splits discussed in the text have been shown. Unless indicated otherwise, taxa were sampled from the South Island of New Zealand.

identifies Fisher's *few petals, coarse hair* group. Group IV, represented by *R. scrithalis*, was not studied by Fisher.

In both the ITS and J_{SA} graphs, groups I–III are separated from each other by moderate to high QP support values. In the ITS graph, which contains outgroup species to root the tree, the relationships between the four groups and outgroups is unresolved, and visually would be represented as a star phylogeny in an unrooted phylogram. There is no evidence in the ITS data to suggest that the outgroups should join with either of the Australian

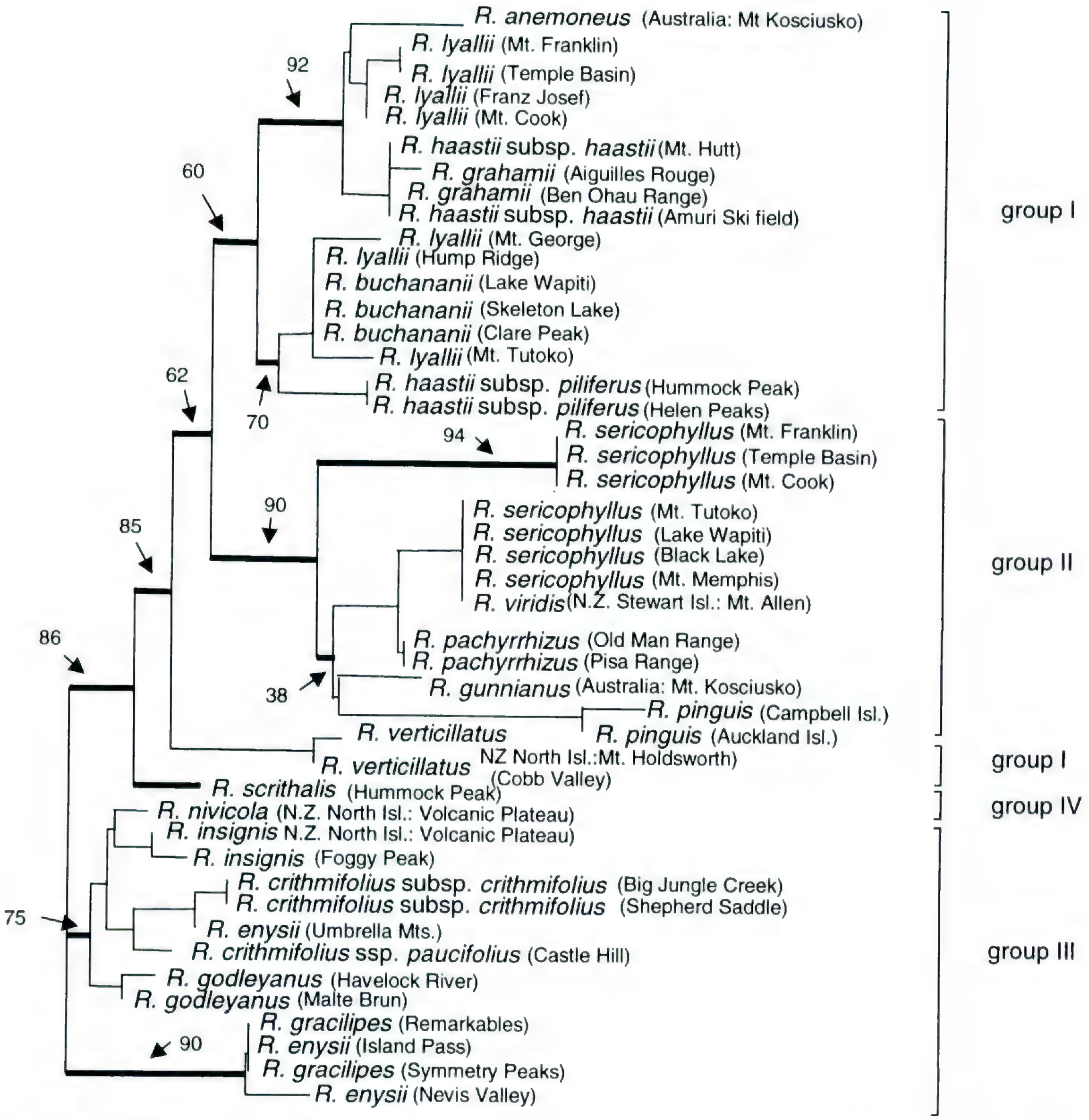


Figure 7. Quartet Puzzle tree for ingroup J_{ST} sequences. Details are the same as described in the legend for Figure 6.

species (0% QP values for outgroups with *R. anemoneus*; 0.3% QP values for outgroups with *R. gunnianus*) or with the subantarctic *R. pinguis* (1.7% QP values for outgroups with *R. pinguis*). Such groupings would be expected if the Australian or subantarctic island species were ancestral to the New Zealand alpine species.

The molecular clock test of Steel et al. (1996) was used to study combined ITS1 and ITS2 data from accessions for taxa corresponding with Fisher's two breeding groups, i.e., *R. buchananii*, *R. haastii* subsp. *haastii*, *R. haastii* subsp. *piliferus*, *R. lyallii*, *R. pachyrrhizus*, *R. sericophyllus*, versus *R. crithmifolius* subsp. *crithmifolius*, *R. crithmifolius* subsp. *paucifolius*, *R. enysii*, *R. gracilipes*, *R. insignis*. All sequences passed the test for a clock-like rate. From these data we obtained a mean point estimate for the age of the last common ancestor for the two breeding groups at 5.01 million years ago (Mya) (i.e., $0.0398088 \pm 0.00664/2 \times 3.94 \pm 0.10$ exp-9 Mya).

PATTERNS OF DIVERSIFICATION WITHIN GROUPS I AND II

The data for groups I and II were examined in detail under split decomposition in Figures 8–11. Comparison of the splits graphs for the chloroplast

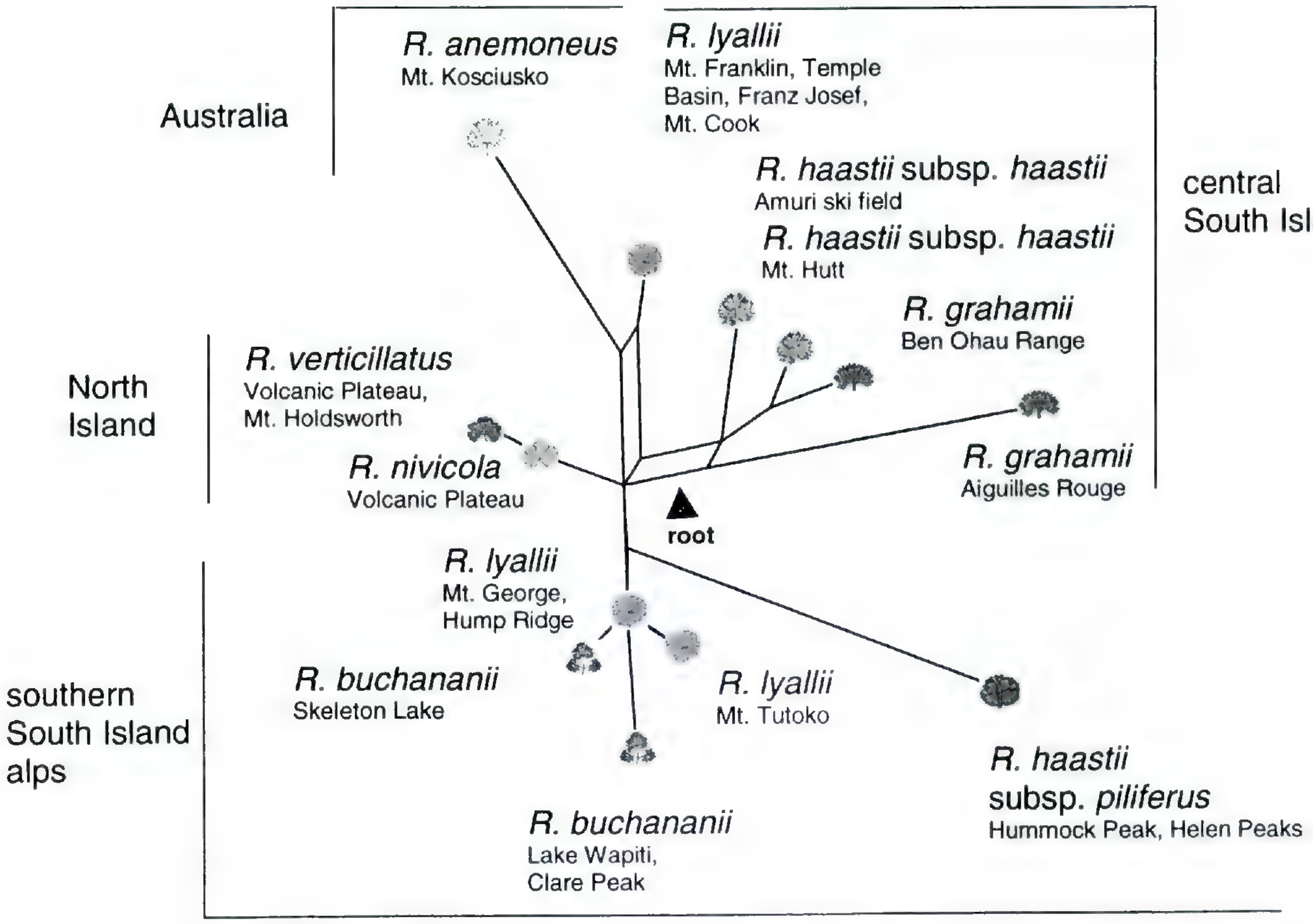


Figure 8. Splits graph of ITS sequences for taxa of group I. The inferred position of the root from Figure 6 is shown for reference. The fit of the splits graph to the ITS sequence data for taxa from group I is very high (Fit = 92).

and nuclear markers shows both tree-like (Fig. 9) and reticulate relationships (Figs. 8, 10, 11). Reticulation is evident between the chloroplast sequences from some of the most diverged species (Fig. 11) and between ITS sequences for some of the most closely related species (Fig. 10). In this latter instance, a complex network describes the relationship between sequences from southern South Island accessions of *R. sericophyllus*, *R. pachyrrhizus*, and *R. viridis* from Stewart Island. Multiple substitutions are one possible explanation for the occurrence of the reticulation between the more diverged chloroplast sequences. However, reticulation between the less diverged ITS sequences is more difficult to explain by the occurrence of multiple substitutions, and the pattern of reticulation may be indicative of hybrid relationships.

A feature of the splits graphs and quartet puzzle trees is that *R. lyallii* and *R. sericophyllus* are paraphyletic. These species have two centers of genetic diversity (i) in the central South Island and (ii) in the southern South Island alps.

Discussion

A monophyletic group

The analyses presented are consistent with the alpine Ranunculi of New Zealand (in the sense of Fisher, 1965; i.e., including the subantarctic *R. pinguis*) being a monophyletic group. This conclusion is supported by ITS sequencing for more widely sampled Australasian species (Armstrong, unpublished data). It is a well circumscribed group of taxa (Fisher, 1965; Webb et al., 1988) and represents section *Pseudadonis* (which includes 18 species; see Table 1). It is a predominantly alpine group, characterized by turgid achenes, simple nectaries, and disproportionately large flowers. Species are polyploid ($2n$ = 48, 96; Hair, 1983; Rendle & Murray, 1989) and linked by hybridization (Fisher, 1965).

A dispersed origin for the founding of an alpine lineage in New Zealand?

Our molecular clock estimates suggest that the primary divergence within the alpine Ranunculi of New Zealand (group III from groups I and II), referred to by Fisher (1965), occurred approximately 5 Mya: a result that would correlate with the onset of Late Tertiary mountain building in New Zealand (Cooper & Millener, 1993; Batt et al., 2000). Prior to this diversification, the evolutionary history of the alpine group is uncertain. The extent of genetic diversity between New Zealand alpine *Ranunculus* species and those from other Southern Hemisphere

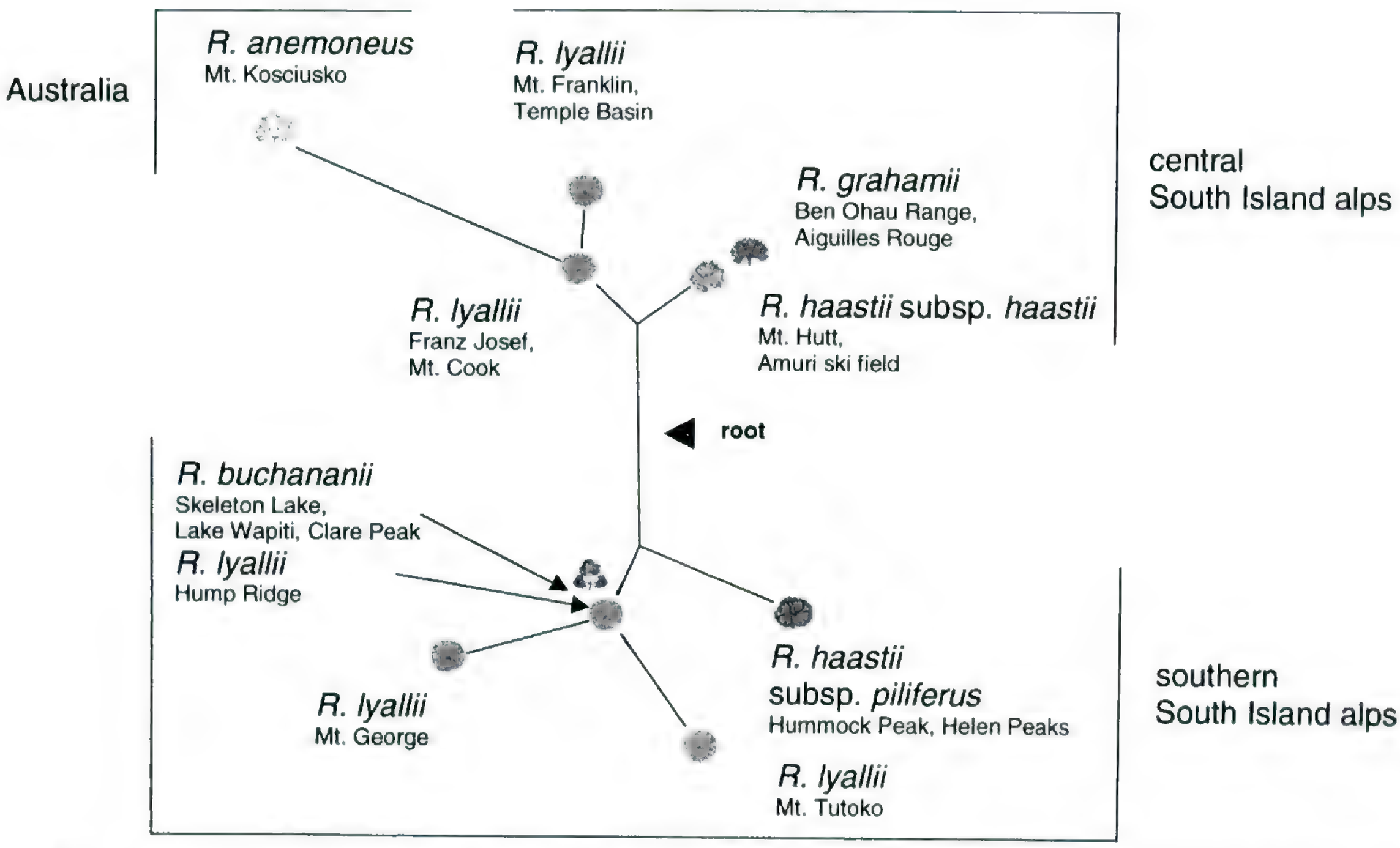


Figure 9. Splits graph of J_{SY} sequences for taxa of group I. The inferred position of the root from Figure 6 is shown for reference. A 9 bp deletion shared by all *R. buchananii* accessions and the *R. lyallii* accessions from Mt. George and Hump Ridge partitions these taxa away from the others in the data set. The fit of the splits graph to the J_{SY} sequence data for taxa from group I is very high (Fit = 100).

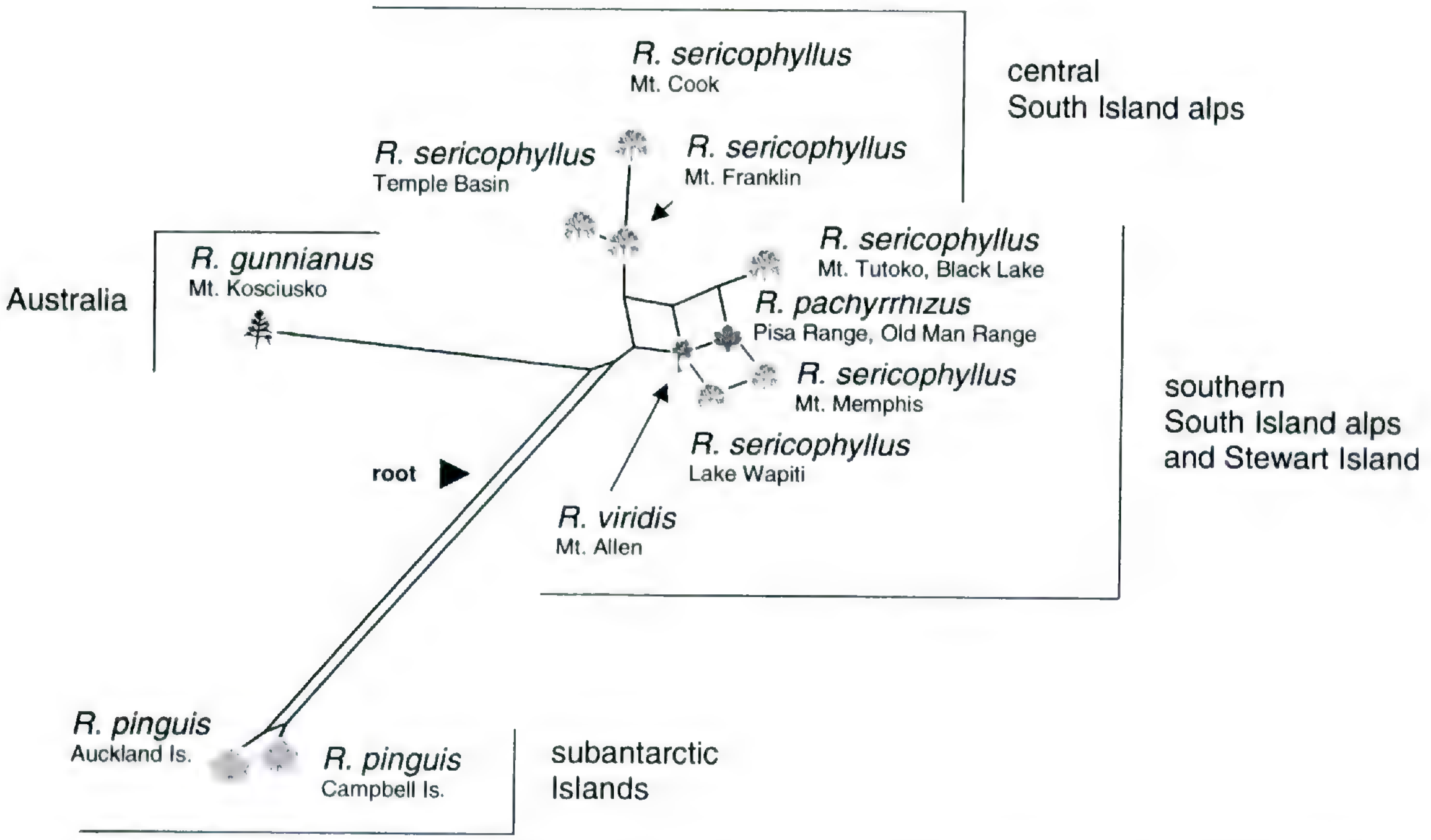


Figure 10. Splits graph of ITS sequences for taxa of group II. The inferred position of the root from Figure 6 is shown for reference. The fit of the splits graph to the ITS sequence data for species from group II is very high (Fit = 100).

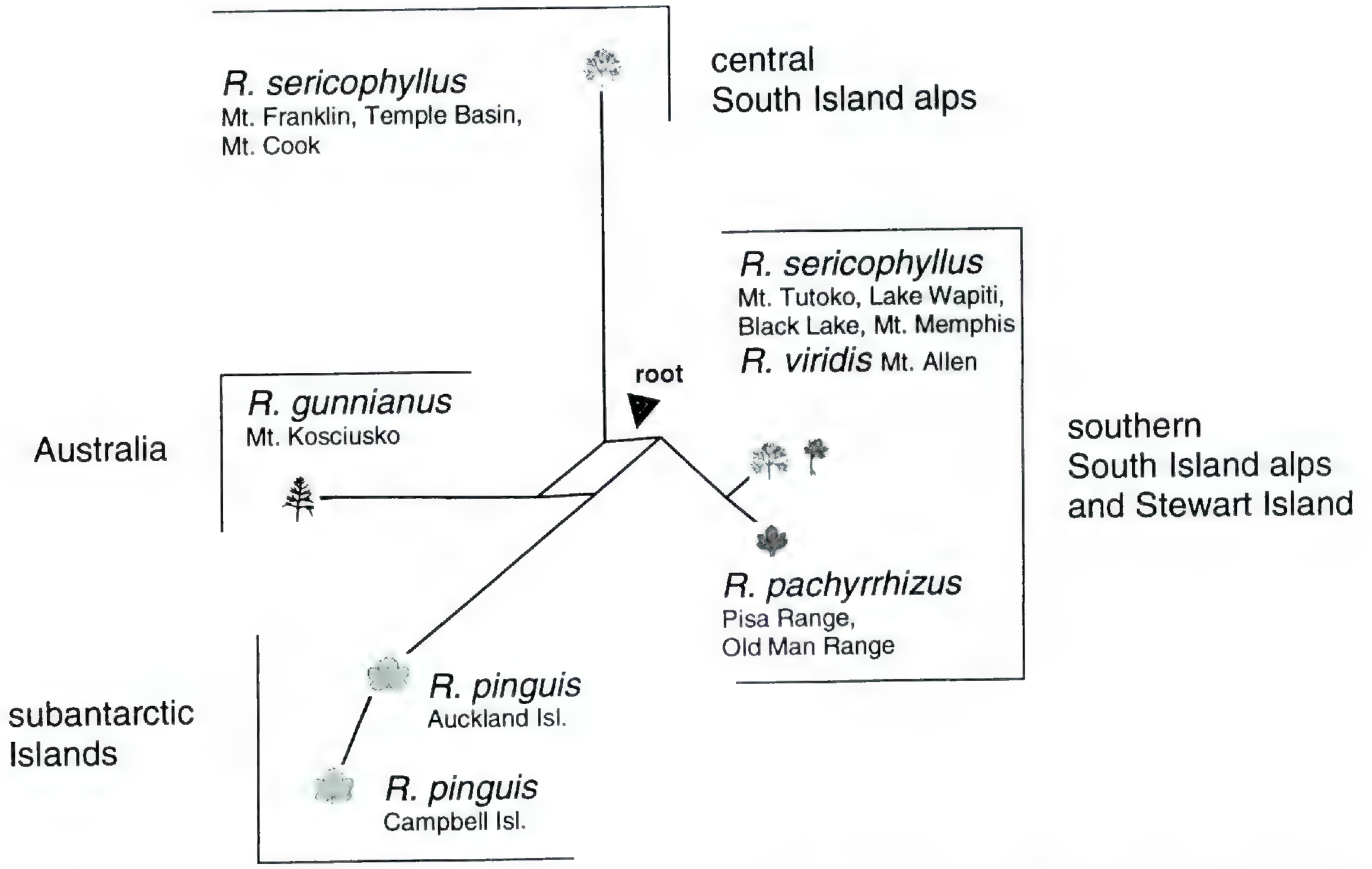


Figure 11. Splits graph of J_{SA} sequences for taxa of group II. The inferred position of the root from Figure 6 is shown for reference. A 9 bp insertion in *R. pinguis* partitions this species from the others in the data set. The fit of the splits graph to the J_{SA} sequence data for taxa from group II is very high (Fit = 100).

lands, as well as from those of the Northern Hemisphere, is too small to suggest that the group has an ancient relic (Gondwanan) distribution in New Zealand. Rather, it seems more likely that the group diversified following transoceanic dispersal of a founding species to New Zealand in the Tertiary.

Chloroplast restriction site data (Johansson, 1998) and unpublished ITS sequence data suggest that closely related to the alpine Ranunculi of New Zealand are worldwide distributed groups such as section *Hecatonia* (*R. sceleratus*), section *Xanthobatrachium* (*R. hyperboreus*), and subgenus *Batrachium* (*R. circinatus*). Two species from these groups were chosen as outgroups in our present study (Fig. 6). Also genetically close to the alpine Ranunculi in New Zealand are the Southeastern Australian species previously investigated by Melville (1955) and the lowland species of section *Epirotes*. These latter species do not appear to be included in section *Pseudadonis* by Tamura (1995), although Tamura does include from this group *R. rivularis* DC. (an earlier illegitimate name for *R. amphitrichus* Colenso: Webb et al., 1988). In Figure 6, this lowland predominantly Australian and New Zealandic group is represented by the coastal species *R. acaulis*. The New Zealand lowland Ranunculi from section *Chrysanthe* are represented here by *R. recens*. This group is more distantly related to the alpine Ranunculi of New Zealand.

It has been suggested (Fisher, 1965; Wardle, 1978; Zimmer & Keener, 1989) that the alpine Ranunculi of New Zealand are derived from South American species or at the least have closest affinity with extant alpine South American species, notably those of section *Trollianthoideae* Lourt. Like the New Zealand species, members of this group occupy alpine habitats and are characterized by disproportionately large flowers and turgid achenes (Fisher, 1965; Tamura, 1995; Lourteig, 1956). An alternative hypothesis has been suggested by Raven (1973). He proposed for many alpine plant genera, including *Ranunculus*, long-distance dispersal to New Zealand via the New Guinea archipelago and Australian mountains.

TRANSOCEANIC DISPERSALS FROM NEW ZEALAND

The hypothesis, that the ancestral alpine *Ranunculus* was originally dispersed to New Zealand, is consistent with the observations in our study that New Zealand has also been the source of transoceanic dispersals for alpine *Ranunculus* to other Southern Hemisphere lands. The Australian alpine

species *R. anemoneus* (strongly clustered within group I) and *R. gunnianus* (strongly clustered within group II) are genetically dissimilar from each other, and neither join with the outgroup species in phylogenetic analyses (e.g., Fig. 6). These observations suggest that the two Australian species are independently derived from within the New Zealand alpine radiation and are not ancestral to it. Similarly, the New Zealand subantarctic species *R. pinguis* (group II) also appears derived from within the New Zealand mainland radiation. In these three cases, dispersal from New Zealand has occurred against prevailing circumpolar westerly winds (Fig. 1), which have existed since the mid Tertiary (Stevens, 1985; Stewart & Neall, 1984). Nevertheless, in the southwestern Pacific frequent anticyclones give rise to easterly wind flows, and it is possible that these may have assisted dispersal in a westward direction (Wardle, 1978; Wallington, 2000).

LOCAL PATTERNS OF SPECIATION AND GLACIAL REFUGIA?

The splits graphs in Figures 8 and 9 indicate that, for group I species, the derivation of high-altitude species with highly restricted distributions has been from ancestral stock of the paraphyletic and geographically widespread *R. lyallii*. In the central South Island alps, i.e., in the northern part of its range (Fig. 2 upper left), *R. lyallii* ancestors appear to have given rise to higher-altitude species such as the scree specialists *R. haastii* subsp. *haastii* and *R. grahamii* of the Mt. Cook region (Figs. 2, 8, 9), both with yellow flowers and winged, curved, smooth achenes. In the southern South Island alps, (Fig. 2), the restricted high-altitude snowline fringe species *R. buchananii* is genetically most similar to southern South Island populations of *R. lyallii* and is inferred to be derived from southern *R. lyallii*-like ancestors (Figs. 8, 9). Note that the southern scree specialist *R. haastii* subsp. *piliferus* is not most closely related to *R. haastii* subsp. *haastii* (Figs. 8, 9), and these taxa presently designated as subspecies may not be monophyletic.

Somewhat in parallel, the splits graphs in Figures 10 and 11 indicate that the derivation of two group II species with highly restricted geographical distributions has been from ancestral stock of the paraphyletic and geographically widespread *R. sericophyllus*. This morphologically and genetically variable species is found along the high elevated ridges of the southern alps from north Canterbury to southwest Otago. The relationships observed in these graphs are consistent with the suggestion by Fisher (1965) that southern South Island *R. sericophyllus*-like species were ancestors of the locally restricted species *R. pachyrrhizus* (Fig. 2). This species has evolved small, coriaceous leaves and has expanded the distribution of its *R. sericophyllus*-like ancestor eastward to central Otago (Fig. 2), a region that has colder and drier winters than the habitat currently occupied by *R. sericophyllus*. *Ranunculus viridis*, native to Stewart Island (Figs. 1, 2), also appears to have originated locally, again from the southern part of the *R. sericophyllus* distribution (Figs. 10, 11).

Further, the splits graphs identify two distinct geographical groupings of genetically most closely related taxa (Figs. 8–11), one in the southern South Island and one in the central South Island. This observation may suggest the existence of two glacial refugia, from which species such as *R. viridis*, *R. pachyrrhizus*, and *R. grahamii* have evolved.

RANGE EXPANSION OF *RANUNCULUS* SECT. *PSEUDADONIS* IN NEW ZEALAND

The South Island southern alps contain most of New Zealand's endemic alpine plant species (McGlone, 1985; Wardle, 1988). Consistent with this pattern, most species of alpine *Ranunculus* also occur in the South Island. It is there that the greatest genetic diversity between species as well as among populations has been identified in our investigations. On the North Island of New Zealand only *R. insignis*, *R. verticillatus*, and *R. nivicola* occur. J_{SA} sequences from *R. insignis* sampled from geographically distant North Island populations have been found to be identical (unpublished obs.). Similarly, North Island ITS sequences for geographically separated populations of *R. verticillatus* have also been found to be very similar (unpublished obs.). If diversification of the four major lineages occurred in the South Island during the Pliocene (within the last 5 My), then the dispersal of both *R. insignis* (from Fisher's *few petals, coarse hair* breeding group) and *R. verticillatus* (from Fisher's *many petals, silky hair* breeding group) into the North Island might have easily occurred during the Pleistocene when snowlines were depressed and a lowering of the sea level by ca. 120 m connected the sounds region of Marlborough with the west coast of the North Island (Fisher, 1965; Te Punga, 1953; Fleming, 1962). During the Pleistocene the South Island of New Zealand was also connected with Stewart Island in the south. Thus the same dispersal mode as for *R. insignis* and *R. verticillatus* might also be suggested for *R. viridis*, a species for which analyses of J_{SA} and ITS sequences suggest

derivation from southern populations of a species ancestral to *R. sericophyllus* (Figs. 10, 11).

An interesting observation is that the North Island distribution of *R. verticillatus* is restricted relative to that of *R. insignis*. Further, the North Island endemic and allopolyploid *R. nivicola* extends the range of these lineages into higher altitudes, as well as into more western and northern North Island alpine regions (Fig. 2). Previously, Fisher (1965) suggested that *R. nivicola* ($2n = 96$) originated via an allopolyploid event between the northward migrating populations of *R. insignis* ($2n = 48$) and *R. verticillatus* ($2n = 48$). The relative position of *R. nivicola* in our ITS and J_{SA} trees (Figs. 6, 7) confirms this hypothesis.

PARALLEL EVOLUTION OF SIMILAR MORPHOLOGIES

Our molecular phylogenies (Figs. 6, 7) provide evidence for a number of convergent morphologies that support Fisher's (1965) argument that the colonization of similar habitats by species from different breeding groups has occurred with the independent evolution of similar adaptations. These include (a) the reflexing of pedicels downward between the leaf blades during ripening of the fruits, a possible adaptation to loose sandy soils, in *R. crithmifolius*, *R. acaulis* (lowland taxon, sect. *Epirotes*), and also in *R. recens* (sect. *Chrysanthe*); (b) stiff petals in species occupying windswept habitats, for example in *R. pinguis* (Campbell and Auckland Islands) and in the distantly related *R. subscaposus* Hook. f. (sect. *Chrysanthe*) of the Auckland Islands (Fisher, 1965); (c) the development of large entire fleshy leaves, as a possible adaptation to low irradiation and high humidity habitats, in *R. pinguis*, *R. lyallii*, and *R. insignis*; and (d) the presence of deep rhizomes, as a possible adaptation to loose screes, in *R. crithmifolius*, *R. scrithalis*, and *R. haastii*.

Convergence in morphology between species even from different genera is a notable feature within the family Ranunculaceae. For example, in the context of the phenotypic states described above, similar structures are recorded from *Anemone blanda* (reflexed pedicels), *Anemonopsis macrophylla* (stiff petals), and *Caltha palustris* (large fleshy leaves) (Ulbrich, 1905). However, note that in other cases, within the alpine Ranunculi of New Zealand, evolution of morphological characters is not convergent and a number of morphological characters are congruent with our molecular phylogeny. These include achene shape, which distinguishes section *Pseudadonis* from section *Chrysanthe*. *Few petals* (5–8) and coarse hairs on leaves, scapes, and achenes are characteristic of species in group III (Figs. 6, 7). *Many petals* (7–16) and silky hairs on leaves, scapes, and achenes characterize species in groups I and II (Figs. 6, 7). However, note the exception of the few-petalled *R. sericophyllus* in this group, which is furnished with three distinct nectary glands per petal suggesting the hypothesis of former fusion of smaller petal primordia (Fisher, 1965). The apparently paraphyletic *R. lyallii* also shares white flowers with its close relatives *R. anemoneus* and *R. buchananii*, but these species (all group I species) do not form a monophyletic clade in our phylogenetic graphs.

SUMMARY

The alpine Ranunculi of New Zealand are a well circumscribed plant group having undergone diversification and range expansion during a period of dramatic geological and climatic change. The molecular analyses we report here confirm many of the hypotheses of Fisher (1965) as well as suggesting some new hypotheses. The observations of reticulation in our ITS splits graphs for diploid species and our confirmation of the allopolyploid nature of *R. nivicola* raises the question whether interspecies hybridization may have been causal in range expansion in a number of instances (Ehrendorfer, 1958; Stebbins, 1984). The origin of the group remains unclear, and this question, as well as the suggested patterns of diversification, needs to be tested with additional intraspecies sampling and higher resolution methods that might better assess the extent of genome introgression (e.g., Wolfe et al., 1998). The approach we have used to find a highly variable molecular marker in the alpine Ranunculi of New Zealand appears useful for obtaining PCR markers for studying other plant groups (e.g., McLenachan et al., 2000). Split decomposition, also used here, has potential for investigating other Late Tertiary–Quaternary radiations. The method is expected to be particularly useful when characterizing potentially hybrid taxa and/or when investigating recently diverged groups. In the present study, we omitted heteroplasmic sites before calculating our phylogenetic graphs, and this general approach is expected to result in loss of valuable information that may indicate hybrid complexity in some taxa. As recently discussed (Huber et al., 2001), a current research interest includes examining how these sites might be incorporated into analyses using both split decomposition and median graphs.

Literature Cited

Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell & M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Ann. Missouri. Bot. Gard. 82: 247–277.

Bandelt, H.-J. 1995. Combination of data in phylogenetic analysis. Pl. Syst. Evol. [Suppl.] 9: 355–361.

——— & A. W. M. Dress. 1992. Split decomposition: A new and useful approach to phylogenetic analysis of distance data. Molec. Phylogenet. Evol. 1: 242–252.

———, P. Forster, B. C. Sykes & M. B. Richards. 1995. Mitochondrial portraits of human populations using median networks. Genetics 141: 743–753.

Batt, G. E., J. Braun, B. P. Kohn & I. McDougall. 2000. Thermochronological analysis of the dynamics of the Southern Alps, New Zealand. Geol. Soc. Amer. Bull. 112: 250–266.

Buckler, E. S., 4th, A. Ippolito & T. P. Holtsford. 1997. The evolution of ribosomal DNA: Divergent paralogues and phylogenetic implications. Genetics 150: 1625–1637.

Cooper, R. A. & P. R. Millener. 1993. The New Zealand Biota: Historical background and new research. Trends Ecol. Evol. 8: 423–461.

Ehrendorfer, F. 1958. Differentiation—Hybridization cycles and polyploidy. Cold Spring Harbor Symposium on Quantitative Biology 24: 141–152.

Fisher, F. J. F. 1965. The Alpine Ranunculi of New Zealand. DSIR Publishing, New Zealand.

Fleming, C. A. 1962. New Zealand biogeography: A paleontologist's approach. Tuatara 10: 53–108.

Golenberg, E. M., M. T. Clegg, M. L. Durbin, J. Doebley & Din Pow Ma. 1993. Evolution of a noncoding region of the chloroplast genome. Molec. Phylogenet. Evol. 2: 52–64.

Hair, J. B. 1983. Contributions to a chromosome atlas of the New Zealand flora. 23. *Ranunculus* (Ranunculaceae). New Zealand J. Bot. 21: 3–7.

Huber, K. T., V. Moulton, P. J. Lockhart & A. Dress. 2001. Lite Median Networks: A technique for studying plant speciations. Molec. Phylogenet. Evol. 19: 302–310.

Huson, D. H. 1998. SplitsTree: Analyzing and visualizing evolutionary data. Bioinformatics 14: 68–73. ⟨ftp.uni-bielefeld.de/pub/math/splits⟩

Johansson, J. T. 1998. Chloroplast DNA restriction site mapping and the phylogeny of *Ranunculus* (Ranunculaceae). Pl. Syst. Evol. 213: 1–19.

Johnson, L. A. & D. E. Soltis. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *mat*K sequences. Ann. Missouri Bot. Gard. 82: 149–175.

Kelchner, S. A. & L. G. Clark. 1997. Molecular evolution and phylogenetic utility of the chloroplast *rpl*16 intron in *Chusquea* and the *Bambusoideae* (Poaceae). Molec. Phylogenet. Evol. 8: 385–397.

Knox, E. B. & J. Palmer. 1995. Chloroplast DNA variation and the recent radiation of giant senecios (Asteraceae) on the tall mountains of eastern Africa. Proc. Natl. Acad. Sci. U.S.A. 92: 10349–10353.

Koch, M. & I. A. Al-Shehbaz. 2000. Molecular systematics of the chinese *Yinshania* (Brassicaceae): Evidence from plastid and nuclear ITS DNA sequence data. Ann. Missouri Bot. Gard. 87: 127–145.

Linder, C. R., L. R. Goertzen, B. V. Heuvel, J. Francisco-Ortega & R. K. Jansen. 2000. The complete external transcribed spacer of 18S–26S rDNA amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely related families. Molec. Phylogenet. Evol. 14: 285–303.

Lockhart, P. J. & P. A. McLenachan. 1997. Isolating polymorphic plant DNA fragments identified using AFLP technology without acrylamide gels: Markers for evolutionary studies. Focus 19: 70–71. ⟨http://www2.lifetech.com/focus_page.html⟩

———, D. Penny & A. Meyer. 1995. Testing the phylogeny of swordtail fishes using split decomposition and spectral analysis. J. Molec. Evol. 41: 666–674.

Lourteig, A. 1956. Ranunculaceas de Sudamerica Tropical. Mem. Soc. Cienc. Nat. La Salle 16: 19–228.

Luo, H. & M. Boutry. 1995. Phylogenetic relationships within *Hevea brasiliensis* as deduced from a polymorphic mitochondrial DNA region. Theor. Appl. Genet. 91: 876–884.

Maier, R., K. Neckermann, G. L. Igloi & H. Koessel. 1995. Complete sequence of the maize chloroplast genome: Gene content, hotspots of divergence and fine tuning of genetic information by transcript editing. J. Molec. Biol. 251: 614–628.

McGlone, M. 1985. Plant biogeography and the late Cenozoic history of New Zealand. New Zealand J. Bot. 23: 723–749.

McLenachan, P. A., K. Stoeckler, R. C. Winkworth, K. McBreen, S. Zauner & P. J. Lockhart. 2000. Markers derived from AFLP gels for plant ecology and evolution studies. Molec. Ecol. 9: 1899–1903.

Melville, R. 1955. Contributions to the flora of Australia: II. Some Ranunculi of Tasmania and South-eastern Australia. Kew Bull. 2: 193–220.

Moulton, V., M. Steel & C. Tuffley. 1997. Dissimilarity maps and substitution models: Some new results. *In* Mathematical Hierarchies and Biology. DIMACS series in Discrete Mathematics and Theoretical Computer Science, Vol. 37, American Mathematical Society.

Olmstead, R. G. & J. A. Sweere. 1994. Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. Syst. Biol. 43: 467–481.

Penny, D., M. D. Hendy, P. J. Lockhart & M. A. Steel. 1996. Corrected parsimony, minimum evolution, and Hadamard conjugations. Syst. Biol. 45: 596–606.

Powell, W., M. Morgante, R. McDevitt, G. G. Vendramin & J. A. Rafalski. 1995. Polymorphic simple sequence repeat regions in chloroplast genomes: Applications to the population genetics of pines. Proc. Natl. Acad. Sci. U.S.A. 92: 7759–7763.

Raven, P. H. 1973. Evolution of subalpine and alpine plant groups in New Zealand. New Zealand J. Bot. 11: 177–200.

Rendle, H. & B. G. Murray. 1989. Chromosome relationships and breeding barriers in New Zealand species of *Ranunculus*. New Zealand J. Bot. 27: 437–448.

Sang, T., D. J. Crawford, S-C. Kim & T. F. Stuessy. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: Evidence from sequences of the ITS regions of nuclear ribosomal DNA. Amer. J. Bot. 81: 1494–1501.

Shan, X., T. K. Blake & L. E. Talbert. 1999. Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor. Appl. Genet. 98: 1072–1078.

Stebbins, G. L. 1984. Polyploidy and the distribution of the arctic–alpine flora: New evidence and a new approach. Bot. Helv. 94: 1–13.

Steel, M. A., M. A Cooper & D. Penny. 1996. Confidence intervals for the divergence between two clades. Syst. Biol. 45: 127–134.

Stevens, G. R. 1985. Lands in collision: Discovering New Zealand's past geography. DSIR Information Series 161.

Stewart, R. B. & V. E. Neall 1984. Chronology of palaeoclimatic change at the end of the last glaciation. Nature 311: 47–48.

Strimmer, K. & A. von Haeseler. 1996. Quartet Puzzling: A quartet maximum likelihood method for reconstructing tree topologies. Molec. Biol. Evol. 13: 964–969.

Swofford, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), Vers. 4. Sinauer, Sunderland, Massachusetts.

———, G. J. Olsen, P. J. Waddell & D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *in* D. M. Hillis, C. Moritz & B. K. Mable (editors), Molecular Systematics, 2nd ed. Sinauer, Sunderland, Massachusetts.

Tamura, M. 1995. Pp. 223–519 *in* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien Band 17 a IV, Angiospermae: Ordnung Ranunculales, Fam. Ranunculaceae, 2nd ed. Duncker & Humblot, Berlin.

Te Punga, M. T. 1953. The Geology of the Rangitikei valley. New Zealand Geol. Surv. Mem. 8: 46.

Ulbrich, E. 1905. Über die systematische Gliederung und geographische Verbreitung der Gattung *Anemone* L. Engler's Botan. Jahrb. 37: 172–256.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper & M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407–4414.

Wallington, J. 2000. West across the Tasman. Austral. Geographic 60: 75–87.

Wardle, P. 1978. Origin of the New Zealand mountain flora, with special reference to trans-Tasman relationships. New Zealand J. Bot. 26: 535–550.

———. 1988. Effects of glacial climates on floristic distribution in New Zealand: 1. A review of the evidence. New Zealand J. Bot. 26: 541–555.

Webb, C. J., W. R. Sykes & P. J. Garnock-Jones. 1988. Flora of New Zealand, Vol. 4. CRI, Landcare Research, New Zealand.

Wolfe, A. D., Q-Y. Xiang & S. R. Kephart. 1998. Diploid hybrid speciation in *Penstemon* (Scrophulariaceae). Proc. Natl. Acad. Sci. U.S.A. 95: 5112–5115.

Yasui, Y. & O. Ohnishi. 1998. Interspecific relationships in *Fagopyrum* (Polygonaceae) revealed by the nucleotide sequences of the *rbc*L and *acc*D genes and their intergenic region. Amer. J. Bot. 85: 1134–1142.

Zimmer, S. N. & C. S. Keener. 1989. A geographical analysis of the family Ranunculaceae. Ann. Missouri Bot. Gard. 76: 1013–1048.

OVERVIEW OF THE NEOTROPICAL GENUS *NOTOPLEURA* (RUBIACEAE: PSYCHOTRIEAE), WITH THE DESCRIPTION OF SOME NEW SPECIES[1]

Charlotte M. Taylor[2]

Abstract

Notopleura (Benth. & Hook. f.) Bremek. is a neotropical genus of about 73 species of herbs and subshrubs, both terrestrial and epiphytic, found in wet neotropical forests from Mexico and the Antilles to Bolivia and Brazil. This genus was formerly included in *Psychotria* L., from which it differs in its low succulent habit, distinctive stipule morphology, usually pseudoaxillary inflorescences, and pyrenes with two germination slits on the adaxial face. Presented here are a description of the morphology of *Notopleura*, a key and enumeration of its species, nomenclature including synonymy and new combinations for 2 subgenera, 53 previously described species, and 4 infraspecific taxa, and descriptions and illlustrations of 18 new species.

Key words: neotropical flora, *Notopleura*, *Psychotria*, Psychotrieae, Rubiaceae.

Notopleura (Benth. & Hook. f.) Bremek. comprises about 73 neotropical species of terrestrial and epiphytic herbs and subshrubs from central Mexico and the Antilles to Brazil and Bolivia. This group was first recognized taxonomically as *Psychotria* sect. *Notopleura* Benth. & Hook. f. in 1874. Bremekamp (1934) first treated this group as a separate genus; subsequently, Steyermark (1972) returned to including this group as a section of *Psychotria* subg. *Heteropsychotria* Steyerm. More recent studies agree that *Notopleura* is after all better treated as a genus separate from *Psychotria* based on both morphological (Taylor, 1996) and molecular (Nepokroeff et al., 1999; Andersson & Rova, 1999) evidence. *Notopleura* is distinguished from *Psychotria* by its low, succulent, often unbranched or clambering habit; its stipules united around the stem into a reduced to well-developed sheath with a single, often glandular, interpetiolar appendage of varied form that is inserted below the top of the sheath; its inflorescences that are pseudoaxillary in most species; and its pyrenes with two small germination slits on the basal end of the adaxial (ventral) face. *Psychotria* does include species with varied habits including a few herbs, species with stipules that are sometimes united around the stem into a continuous sheath, and species with sometimes pseudoaxillary inflorescences. However, stipules with a medial appendage are unique to *Notopleura*.

Here I review the morphology of *Notopleura*; provide a list (see Appendix 1) of its component species and infrageneric classification and keys to both of these; make the necessary nomenclatural combinations to separate *Notopleura* from *Psychotria*; and describe several new species. *Notopleura* has not been treated in its entirety since Bremekamp (1934) included 4 species. Here 2 new subgenera are recognized and 69 species are added. In general, members of subgenus *Notopleura* are terrestrial and have pseudoaxillary inflorescences, while those of subgenus *Viscagoga* are epiphytes with inflorescences that may be terminal or pseudoaxillary. About a fourth of the *Notopleura* species are newly described in this article; these are variously distributed from Guatemala through Bolivia.

Morphology of *Notopleura*

Plants of *Notopleura* are rather succulent, usually unbranched or little-branched herbs or subshrubs 1 m tall or shorter, though a few species may reach 3 m tall. The terrestrial species (subg. *Notopleura*) are found in the understory of wet forest, most commonly in wet microsites such as swamps and stream edges, while the epiphytic spe-

[1] I thank the curators of BM, BR, COL, CUVC, F, FMB, GB, HUA, JAUM, K, NY, S, TULV, and WIS for loan of or access to specimens; R. E. Gereau for help with the Latin diagnoses; and D. Lorence, L. Andersson, M. Correa, M. Nepokroeff, and M. Grayum for comments and help with additional information.

[2] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A.

cies (subg. *Viscagoga*) are found in wet forest canopy or sometimes in the subcanopy near light gaps. The vegetative portions of the plants are usually glabrous but may be puberulous, pilosulous, or villosulous with rather short trichomes, or in a few species, notably *N. lanosa*, they may be densely villous with trichomes 1–2.5 mm long. Both vegetative and reproductive tissues contain raphides that are usually densely distributed relative to most Psychotrieae, and easily visible in dried specimens.

The leaves are opposite, and in subgenus *Notopleura* often have well-developed petioles and relatively large blades, in some species to 40 cm long. The succulent leaves may have a tough or more often a thin delicate texture when dry. In life they are often silvery or dark and velvety on one or both surfaces, and frequently the abaxial (i.e., lower) leaf surface is paler or shinier than the adaxial surface. The secondary veins are usually visible, and are usually plane on the adaxial surface and prominulous on the abaxial surface; no domatia have been found associated with them, or elsewhere on the leaves. In several species the secondary veins unite in a clear submarginal collecting vein (e.g., Fig. 5D). Higher-order leaf venation is usually not readily visible in any species of *Notopleura*, and in a few species of both subgenera the secondary veins are not visible either (e.g., Figs. 2A, 2H).

The stipules of species of *Notopleura* in part distinguish this genus: they are united around the stem (and sometimes also to the petioles), into a sheath that bears in the center (i.e., medially) of each interpetiolar side a distinctive appendage. This stipule sheath may be reduced to a line but is usually well developed with a truncate to broadly triangular outline, and may be caducous or persistent. The interpetiolar appendages vary widely in form, and are sometimes distinctive for a particular species. These appendages may be well developed to reduced or in a few species (e.g., *N. congesta* C. M. Taylor) apparently secondarily absent, and either caducous or persistent. In most species of both subgenera, the stipule sheath is rather well developed and the appendage is conical to linear, 3 mm long or shorter, succulent, divergent from the stem, and entire to shortly bifid, bilobed, or fimbriate at the apex with the adaxial surface or the apical projections usually glandular (e.g., Figs. 3A, 3H, 4A, 6A, 6D). In some species this interpetiolar appendage is expanded into a membranaceous to papyraceous, lanceolate to ovate or elliptic lamina. This laminar appendage is often evidently medial in its insertion in the sheath, but in some species the sheath is reduced to a line of tissue and the lamina appears terminal on it or even directly connected to the petioles (e.g., Fig. 2E). The margins of these laminar appendages are typically slightly to strongly erose or laciniate, with the marginal projections often apparently glandular. In many species of subgenus *Notopleura*, the stipule appendages are very succulent and consequently on dried specimens their shape may be difficult to discern. Thus although stipule form is useful for distinguishing species of *Notopleura*, it has been little used in this group and herbarium specimens are often not very informative. The stipule descriptions presented here are based almost exclusively on herbarium specimens, so certainly study of living plants will reveal additional features.

The inflorescences of *Notopleura* are terminal or pseudoaxillary (i.e., they are produced in a terminal position developmentally but the stem grows from a developmentally axillary bud, so the inflorescence is found in only one axil at each node). In subgenus *Viscagoga*, the inflorescences are pedunculate and paniculate with several orders of branching, and the flowers are pedicellate in few-flowered cymules. In subgenus *Notopleura*, the inflorescences vary from sessile to long-pedunculate, and from capitate (e.g., Figs. 4D, 6J) to paniculiform with several degrees of branching (e.g., Figs. 1H, 2A). The flowers may be pedicellate but are more often sessile or subsessile, and the fruits are also usually sessile but sometimes subsequently develop a stipitate basal extension as they mature (e.g., Fig. 3A). The flowers and fruits may be distributed along the axes singly or in small glomerules (e.g., Fig. 3I), or they may be grouped into larger glomerules that are all apical on the axes (e.g., Fig. 5D, 5I). The inflorescence axes are usually dichasial in development at least at lower nodes, though in many species they become monochasial or cincinnoid later in development or in higher-order axes, with the flowers or glomerules alternate and well separated along them. Branched inflorescences have two or frequently three axes at the branching nodes; this latter condition is unusual in the Rubiaceae. The inflorescence axes are usually subtended by bracts that are situated at the branching nodes. The floral glomerules are also usually subtended by bracts, and the individual flowers are usually subtended by floral bracts (i.e., bracteoles). The capitate inflorescences appear to have an arrangement similar to that found in the branched inflorescences, except with the bracts usually exceptionally well developed and the axes not or hardly developed. In several species the inflorescences are capitate at anthesis but expand as the fruits develop (e.g., Fig. 2H).

The flowers are similar among species of *Noto-*

pleura in their relatively small size and usually white corollas, and are apparently diurnal and insect-pollinated. Some species are distylous[3] but others appear to be monomorphic. The flowers have not been described in detail or used to separate species by most previous authors, but they do present several distinctive characters, as noted below. No published pollination studies have been found for *Notopleura*, and no odors or floral visitors have been noted in the collection data for the specimens studied.

The hypanthium (i.e., ovary) portion of the flowers is turbinate to cylindrical or ellipsoid and usually about 1 mm long at anthesis. This structure is generally similar among the species of *Notopleura*, while the calyx limb (i.e., the free portion of the calyx that extends from the top of the hypanthium, also sometimes called the calyx cup or simply the calyx) is more variable in form (e.g., Figs. 1F, 2C, 2G, 4B). The calyx limb usually has a short tubular portion, usually 1 mm long or shorter, and a margin that varies from truncate to deeply four- or five-lobed, with the lobes as much as 6 mm long.

The corollas of *Notopleura* are funnelform to somewhat tubular, white or rarely orange to yellow (e.g., *N. marginata* (Benth.) Bullock), and five-lobed, or in some species of subgenus *Viscagoga* four-lobed, or infrequently six-lobed in occasional anomalous flowers of various species. The corolla lobes are valvate, and those of several species are thickened on their abaxial surfaces (e.g., Figs. 3B, 6H); in some species, these thickenings may be prolonged into pronounced appendages (e.g., Figs. 2D, 5E). Externally, the corolla may be glabrous or pubescent throughout, with the pubescence often less or more dense on the lobes, or infrequently markedly denser only on the corolla lobe appendages. This external pubescence pattern apparently varies within as well as among species. Internally the corolla may be glabrous throughout or more often has a pubescent ring at or above the middle of the tube or sometimes in the throat; the internal pubescence pattern appears to be consistent within species.

The stamens are five, or four in flowers with four corolla lobes, and are inserted at or above the middle of the corolla tube with the filaments very short to well developed. The anthers are narrowly oblong, and may be included or exserted. The stigmas are two, four, or rarely six (e.g., some plants of *N. epiphytica* (K. Krause) C. M. Taylor), linear, and positioned variously from near the middle of the corolla tube to well exserted. The style is surrounded at the base by a low annular disk. Distyly is predominant in Psychotrieae (Taylor, 1996), and is certainly present in many species of *Notopleura* (e.g., *N. aggregata* (Standl.) C. M. Taylor, Monteverde Reserve, Costa Rica, pers. obs.) with flowers that show reciprocal positioning of anthers and stigmas with usually only one of these exserted. However, the presence of distyly seems unlikely in some species of *Notopleura*, which on herbarium specimens have the anthers and stigmas both exserted and positioned at nearly the same level (e.g., *N. scarlatina* C. M. Taylor). Overall, relatively few herbarium collections of any species of *Notopleura* have flowers at anthesis in good condition, so these conclusions are provisional.

The fruits of *Notopleura* are spongy or succulent drupes about 1–1.5 cm in diameter, which are assumed to be dispersed by birds. At maturity the fruits may be white, orange, red, or black. In some species (e.g., *N. polyphlebia* (Standl.) C. M. Taylor, *N. uliginosa* (Sw.) Bremek.) the drupes pass through rather orange or red immature stages and then quickly become black, apparently overnight, and are rapidly removed by frugivores, so care must be taken in determining mature fruit color. For some species the fruit color has been reported as variable, but mature fruit color is here considered generally consistent within a species and thus taxonomically informative. In particular, Steyermark (1972) included in *N. macrophylla* (Ruiz & Pav.) C. M. Taylor plants with white, yellow-green, and red fruits, but here *N. macrophylla* is restricted to plants with white fruits. The fruits of *Notopleura* contain two, four, or rarely six (e.g., *N. epiphytica*) pyrenes. When the pyrenes number four to six they are triangular in cross section with two adaxial (i.e., ventral) faces; when they number two, they are hemispherical or dorsiventrally flattened in cross section with only one adaxial face. On the adaxial face or faces the pyrenes may be smooth or may

[3] Distyly (heterostyly with two style lengths) is a common condition in the Rubiaceae, in which flowers of two forms (morphs) are found in a single species: in the short-styled form (thrum) the styles are relatively short so the stigmas are positioned below the anthers, and the anthers are usually exserted or positioned at the corolla throat on well-developed filaments; in the long-styled form (pin) the styles are well developed so the stigmas are exserted or positioned in the corolla throat, and the anthers are positioned below them on relatively short filaments. Typically the positions of the anthers and stigmas in one floral form correspond to the positions of the stigmas and anthers, respectively, in the other floral form. Pollination is thus preferentially between floral forms rather than among flowers of the same form, and this preference is reinforced (in the taxa so far studied) by an incompatability system. An individual plant bears flowers of only one form, and usually long-styled and short-styled plants are found in equal proportions in the population of a given area.

have one longitudinal central ridge or two to three shallow longitudinal depressions; on the abaxial face the pyrenes may be smooth or ridged, in the latter case with one central ridge or three to five smaller ridges and the margins also sometimes thickened. The pyrenes open through two small germination slits near the basal end, a characteristic that is apparently unique in the tribe and in part distinguishes *Notopleura* (Nepokroeff, 1997).

Taxonomic Treatment

The following treatment is based on review of literature and specimens from the herbaria listed in the acknowledgments. The species in each subgenus are presented in alphabetical order. *Notopleura standleyana* (Steyerm.) C. M. Taylor of Venezuela is not included in the key to species of subgenus *Notopleura* because its original description did not include adequate information on the characters here considered distinctive, and the specimens seen of this species are inadequate for determination of this missing information.

Notopleura (Benth. & Hook. f.) Bremek., Rec. Trav. Bot. Néerl. 31: 289. 1934. *Psychotria* sect. *Notopleura* Benth. & Hook. f., Gen. Pl. 2: 124. 1874. TYPE: *Notopleura marginata* (Benth.) Bullock.

Plants rather succulent, terrestrial (subg. *Notopleura*) or epiphytic (subg. *Viscagoga*) herbs or subshrubs; stems branched or unbranched, erect or sometimes climbing, clambering, or reclining. Leaves paired, petiolate, decussate, without domatia; stipules united around the stem and/or to the petioles into a sheath usually bearing an interpetiolar appendage or rarely this absent, when present the appendage succulent, persistent or caducous, medial, conical to linear, entire to bilobed or apically fimbriate, and usually glandular, or less often the appendage laminar, membranaceous to papyraceous, medial or terminal, elliptic to ovate, and entire to erose or laciniate (subg. *Notopleura*). Inflorescences terminal (subg. *Viscagoga*) or pseudoaxillary (subg. *Viscagoga*, subg. *Notopleura*), sessile to pedunculate, capitate to paniculiform, bracteate; flowers distylous or apparently sometimes monomorphic; calyx limb 5(6)-lobed or occasionally 4-lobed (subg. *Viscagoga*); corolla funnelform to somewhat tubular, externally and internally glabrous or variously pubescent, lobes 5(6) or occasionally 4 (subg. *Viscagoga*), valvate in aestivation, sometimes abaxially thickened or appendaged; stamens 5 or occasionally 4 (subg. *Viscagoga*), inserted in corolla tube, anthers exserted or included; stigmas 2 or sometimes 4 (subg. *Viscagoga*) or rarely 6 (subg. *Viscagoga*), linear, exserted or included, style surrounded at base by an annular disc. Fruit fleshy, drupaceous, at maturity orange, red sometimes becoming black at maturity, or white; pyrenes 2 to 6, when 3 to 6 then triangular in cross section, when 2 then hemispherical to dorsiventrally flattened in cross section, on the abaxial face smooth or with 1 to 5 longitudinal ridges and the margins sometimes also thickened, on the adaxial face smooth, with a central longitudinal ridge, or with 1 to 3 shallow longitudinal depressions.

About 62 species, Mexico and the Antilles to the Guianas, Brazil, and Bolivia, in wet formations from sea level to montane forest. The typification of this genus is discussed by Bullock (1958).

Key to the Subgenera of *Notopleura*

1a. Plants terrestrial, usually unbranched, usually erect; stipules with interpetiolar appendage succulent, or expanded into a lamina, or absent; inflorescences pseudoaxillary; pyrenes 2, in cross section hemispherical to strongly dorsiventrally flattened ..... subg. *Notopleura*

1b. Plants epiphytic, usually branched, often clambering or climbing; stipules with interpetiolar appendage succulent or absent; inflorescences terminal or pseudoaxillary; pyrenes 2 to 6, in cross section hemispherical to triangular or somewhat dorsiventrally flattened ..... subg. *Viscagoga*

Notopleura subg. **Notopleura**

Montamans Dwyer, Ann. Missouri Bot. Gard. 67: 285. 1980. TYPE: *Montamans panamensis* Dwyer ≡ *Notopleura panamensis* (Dwyer) C. M. Taylor.

Plants terrestrial; stems usually unbranched, generally erect. Stipules with interpetiolar appendages succulent and conical to ovate or linear, or expanded into a membranaceous to papyraceous lamina, or rarely absent. Inflorescences pseudoaxillary; calyx limb 5-lobed; corolla 5(6)-lobed, the lobes sometimes horned or appendaged. Fruit yellow, orange, red sometimes becoming black at maturity, or white; pyrenes 2, in cross section hemispherical to dorsiventrally flattened, on the abaxial face smooth or with 1 to 5 longitudinal ridges, on the adaxial face smooth or with 1 central longitudinal ridge or 1 to 3 shallow longitudinal depressions.

About 52 species, Mexico and the Antilles to the Guianas, Brazil, and Bolivia, in wet formations from sea level to upper montane forest.

KEY TO THE SPECIES OF *NOTOPLEURA* SUBG. *NOTOPLEURA*

1a. Leaves with margins markedly thickened and cartilaginous; calyx limbs 3–4 mm long ..... *N. macropodantha*
1b. Leaves with margins membranaceous to cartilaginous but not markedly thickened; calyx limbs 0.2–4 mm long.
2a. Inflorescences at anthesis capitate to subcapitate with the axes not visible, sometimes expanding as fruits develop.
3a. Stems, petioles, stipules, and leaves on both surfaces lanose to villous with trichomes 1–2.5 mm long ..... *N. lanosa*
3b. Stems, petioles, stipules, and leaf surfaces glabrous or sometimes puberulous to pilosulous, hirtellous, or villosulous with trichomes < 1 mm long.
4a. Inflorescences and infructescences sessile or with peduncles shorter than or equal to petioles.
5a. Mature pyrenes smooth on abaxial (i.e., dorsal) surface.
6a. Stipules with sheaths developed, truncate to broadly rounded, without interpetiolar appendages; calyx limbs 1–1.5 mm long ..... *N. congesta* (p.p.)
6b. Stipules with sheaths reduced, with appendages expanded and laminar; calyx limbs 2–4 mm long.
7a. Stipules 2–5 mm long, laciniate with the segments often terminating in filaments; French Guiana ..... *N. lateralis*
7b. Stipules 5–15 mm long, erose to deeply so, the segments acute but usually not terminating in filaments; Central America and western South America.
8a. Leaves drying subcoriaceous to chartaceous, with the secondary veins not visible on the abaxial surface; Costa Rica to Panama ..... *N. camponutans*
8b. Leaves drying papyraceous, with the secondary veins visible on the abaxial surface; Panama to Peru ..... *N. plagiantha*
5b. Mature pyrenes with 1 to 3 longitudinal ridges on abaxial surface.
9a. Stipules with sheaths reduced and lanceolate to ovate or elliptic interpetiolar laminas 5–20 mm long.
10a. Stipules persistent with the leaves ..... *N. latistipula*
10b. Stipules caducous before the leaves.
11a. Leaves with secondary veins 13 to 17 pairs ..... *N. araguensis*
11b. Leaves with secondary veins 10 to 12 pairs ..... *N. thesceloantha*
9b. Stipules with sheaths developed, truncate to triangular, 0.5–5 mm long, with interpetiolar appendages absent or narrowly triangular to linear or conical.
12a. Leaves with secondary veins not or hardly visible on the abaxial surface; inflorescences sessile or subsessile, with the peduncles elongating as the fruits develop ..... *N. iridescens* (p.p.)
12b. Leaves with secondary veins clearly visible on the abaxial surface; inflorescences sessile or pedunculate, the peduncles usually not elongating as the fruits develop.
13a. Inflorescences enclosed by 2 to 6 ovate to lanceolate, involucral bracts 5–20 mm long; fruits orange or red becoming black at maturity.
14a. Plants glabrous or usually villosulous or pilosulous; leaves elliptic to oblanceolate, 3.5–5 cm wide, at apex sharply acute to acuminate and rather tapered ..... *N. acuta*
14b. Plants glabrous or puberulous; leaves elliptic to broadly so or elliptic-oblong, 5–15 cm wide, at apex broadly rounded to obtuse, acute, or deltoid, sometimes shortly acuminate but not tapered ..... *N. leucantha* (p.p.)
13b. Inflorescences with numerous lanceolate to ovate bracts 2–8 mm long, these external but not involucral; mature fruits red or white.
15a. Calyx limbs 2.5–4 mm long.
16a. Corolla lobes abaxially with appendages 3.5–4 mm long ..... *N. panamensis*
16b. Corolla lobes abaxially smooth ..... *N. uberta*
15b. Calyx limbs 0.5–2 mm long.
17a. Fruits red at maturity ..... *N. palestinae* (p.p.)
17b. Fruits white at maturity.
18a. Floral bracts lanceolate, 8–10 mm long; Venezuela ..... *N. decurrens*
18b. Floral bracts lanceolate to ovate, 2–5 mm long; Nicaragua to northwestern Colombia ..... *N. tonduzii*
4b. Inflorescences and infructescences with peduncles longer than petioles.
19a. Leaves plane or often bullate, with secondary veins prominulous on adaxial surface ..... *N. polyphlebia*
19b. Leaves plane, with secondary veins plane on adaxial surface.

20a. Leaves with secondary veins not or hardly visible on abaxial surfaces; pyrenes dorsiventrally flattened, on abaxial face with 1 or 3 longitudinal ridges ..... *N. iridescens* (p.p.)

20b. Leaves with secondary veins clearly visible on abaxial surfaces; pyrenes hemispherical in cross section, on abaxial face smooth or with 1 longitudinal ridge.

21a. Pyrenes dorsiventrally flattened in cross section, on abaxial face with a longitudinal ridge; inflorescences hemispherical to depressed-globose ..... *N. leucantha* (p.p.)

21b. Pyrenes hemispherical in cross section, on abaxial face smooth; inflorescences pyramidal to cylindrical or ovoid ..... *N. pyramidata*

2b. Inflorescences at anthesis branched with the axes clearly evident.

22a. Stipules with sheaths reduced and interpetiolar appendages expanded into laminas that are broader than the stem diameter; pyrenes on abaxial face with 3 to 5 longitudinal ridges, or smooth in *N. callejasii*.

23a. Inflorescences sessile or with peduncles to 1.5 cm long, the peduncles shorter than the principal axes of the inflorescence.

24a. Floral bracts acute to obtuse; calyx limbs ca. 2 mm long ..... *N. micayensis*

24b. Floral bracts obtuse to rounded or truncate; calyx limbs ca. 1 mm long .... *N. wilburiana*

23b. Inflorescences with peduncles 2.5–25 cm long, the peduncles equal to or usually longer than the principal axes of the inflorescence.

25a. Leaves drying subcoriaceous to chartaceous, with the secondary veins not or hardly visible on the abaxial surface.

26a. Inflorescence bracts oblong to elliptic or lanceolate, those subtending the basalmost secondary axes equal to or longer than the first internodes of these axes; Venezuela ..... *N. sucrensis*

26b. Inflorescence bracts narrowly triangular or narrowly ligulate to linear, those subtending the basalmost secondary axes shorter than the first internodes of these axes; western Colombia and Ecuador.

27a. Calyx limbs ca. 0.8 mm long; Ecuador ..... *N. albens*

27b. Calyx limbs 3–4.5 mm long; Colombia ..... *N. callejasii*

25b. Leaves drying papyraceous to membranaceous, with the secondary veins visible on the abaxial surface.

28a. Inflorescences in profile corymbiform-rounded, with their basalmost nodes producing 2 or usually 3 to 4 secondary axes, these all generally equal in length to the primary axis; corollas orange to yellow or sometimes white on tube ..... *N. marginata*

28b. Inflorescences in profile pyramidal to rounded or cylindrical, with their basalmost nodes producing 2 or sometimes 3 secondary axes that are clearly shorter than the primary axis; corollas white.

29a. Inflorescence bracts lanceolate to narrowly triangular, equal to or longer than the flower buds but narrow and leaving visible the buds, the calyx limbs at anthesis, and the developing fruits ..... *N. longipedunculata*

29b. Inflorescence bracts elliptic to ovate, broad enough to completely cover the flower buds, the calyx limbs at anthesis, and the developing fruits.

30a. Calyx limbs 3–4 mm long; secondary leaf veins eucamptodromous ..... *N. longissima*

30b. Calyx limbs 1–2 mm long; secondary leaf veins brochidodromous.

31a. Secondary leaf veins 9 to 13 pairs; peduncles 2.5–9 cm long ..... *N. dukei*

31b. Secondary leaf veins 18 to 22 pairs; peduncles 6–29 cm long ..... *N. lateriflora*

22b. Stipules with sheath developed, truncate to rounded or broadly pyramidal and interpetiolar appendage lacking or usually present, conical to lanceolate, obovate, or ligulate, narrower than the diameter of the stem; pyrenes on abaxial face smooth or with 1 to 5 longitudinal ridges.

32a. Leaves truncate at base; petioles 0.5–1 cm long ..... *N. subimbricata*

32b. Leaves acute to obtuse or caudate at base; petioles 1–15 cm long.

33a. Mature pyrenes in cross section hemispherical to triangular and inflated, abaxially smooth or with 1 to 5 low longitudinal ridges and the margins not or somewhat thickened.

34a. Fruits red to orange at maturity (color unknown in *N. cundinamarcana*); mature pyrenes smooth abaxially.

35a. Stipule sheaths 3–7 mm long.

36a. Young stems glabrous or puberulous with the trichomes appressed.

37a. Peduncles 1–8.5 cm long; flowering portion of inflorescence 2.5–4.5 × 2.5–6 cm; calyx limbs 2.5–5 mm long; corolla tubes 5–6 mm long ..... *N. cundinamarcana*

37b. Peduncles 0.7–1.1 cm long; flowering portion of inflorescence

1.5 × 2–2.5 cm; calyx limbs ca. 0.5 mm long; corolla tubes 1.5–2 mm long ..... *N. saulensis*

36b. Young stems pilosulous or hirtellous with spreading trichomes.

38a. Inflorescences with floral bracts 0.5–1.2 mm long, rounded to obtuse, incompletely enclosing the flower buds ..... *N. pilosula*

38b. Inflorescences with floral bracts 2–5 mm long, acute to obtuse, completely enclosing the flower buds ..... *N. siggersiana*

35a. Stipule sheaths 0.5–2.5 mm long.

39a. Inflorescences with peduncles 8–23 cm long; infructescences 14–22 cm wide at widest point; Guatemala and Honduras ..... *N. hondurensis*

39b. Inflorescences with peduncles 1–11 cm long; infructescences 1.5–12 cm wide at widest point; eastern Panama to Peru.

40a. Corolla lobes abaxially smooth; peduncles 2–11 cm long; branched portions of inflorescences 2.5–11 cm long.

41a. Leaves 3–5.5 cm wide; eastern Panama ..... *N. angustissima*

41b. Leaves 8–12.5 cm wide; Ecuador ..... *N. scarlatina*

40b. Corolla lobes abaxially with short conical to linear appendages, these evident at least in bud; peduncles 0.4–5.5 cm long; branched portions of inflorescences 1–4.5 cm long.

42a. Stipules without interpetiolar appendages ... *N. congesta* (p.p.)

42b. Stipules with interpetiolar appendages.

43a. Inflorescences with branched portions 0.6–1.8 cm long; stipule sheaths 2–3 mm long; central Panama to western Colombia ..... *N. palestinae* (p.p.)

43b. Inflorescences with branched portions 1–4.5 cm long; stipule sheaths 0.5–1.5 mm long; Amazonian Colombia to Peru ..... *N. parasiggersiana*

34b. Fruits white at maturity (color unknown in *N. humensis*); mature pyrenes abaxially 1 to 5 longitudinal ridges.

44a. Leaves 3–5.5 cm wide; inflorescences with branched portions 1–2 cm long; corolla lobes abaxially with appendages; French Guiana ..... *N. microbracteata*

44b. Leaves 2–15 cm wide; inflorescences with branched portions 2–15 cm long; corolla lobes abaxially smooth or with appendages; Mexico and the Antilles to Bolivia and the Guianas.

45a. Corolla lobes abaxially with appendages; Jamaica and the Lesser Antilles ..... *N. discolor*

45b. Corolla lobes abaxially smooth; Mexico and Central America to central South America.

46a. Leaves elliptic to broadly elliptic or elliptic-oblong, with at least some secondary veins extending to and uniting with the margins; peduncles 1–7 cm long; mature pyrenes triangular to somewhat inflated in cross section ..... *N. macrophylla*

46b. Leaves narrowly elliptic to oblanceolate or lanceolate, with secondary veins weakly brochidodromous; peduncles 3–16 cm long; mature pyrenes hemispherical in cross section ..... *N. tolimensis*

33b. Mature pyrenes in cross section dorsiventrally markedly flattened, abaxially with 1 to 3 longitudinal ridges and the margins smooth to thickened.

47a. Leaves narrowly elliptic, with secondary veins not visible on the abaxial surface.

48a. Leaves tapering to a long-acute or attenuate base; bracts subtending the secondary inflorescence axes equal to or usually much shorter than the first internodes of the secondary axes ..... *N. tapajozensis*

48b. Leaves tapering to an obtuse or abruptly truncate base; bracts subtending the secondary inflorescence axes equal to or usually longer than the first internodes of the secondary axes.

49a. Leaf bases rounded to truncate; bracts subtending the secondary inflorescence axes 17–30 × 5–14 mm ..... *N. aneurophylla*

49b. Leaf bases obtuse; bracts subtending the secondary inflorescence axes 15–18 × 8 mm ..... *N. aneurophylloides*

47b. Leaves narrowly to broadly elliptic, obovate, or oblanceolate, with secondary veins visible on abaxial surface.

50a. Leaves with secondary veins 30+ pairs, prominulous on the adaxial surface ..... *N. agostinii*

50b. Leaves with secondary veins 30 or fewer pairs, impressed to plane or sometimes a little thickened on the adaxial surface.

51a. Leaves with secondary veins impressed on both surfaces .... *N. chapensis*

51b. Leaves with secondary veins plane to thickened on both surfaces.

52a. Leaf bases decurrent on the petiole ..... *N. humensis*
52b. Leaf bases not decurrent on the petiole.
53a. Mature fruits orange, red, or black.
54a. Leaves pilosulous to villosulous on the abaxial surface; inflorescences with 2 or usually 3 secondary axes at the basal node.
55a. Leaves 8–16 cm long; inflorescences with branched portions 1–3 cm long; pyrenes 6–8 × 4.5–5.5 mm ..... *N. aequatoriana*
55b. Leaves 9–32 cm long; inflorescences with branched portions 5.5–13 cm long; pyrenes 4–5.5 × 4–5 mm ..... *N. triaxillaris*
54b. Leaves glabrous to puberulous on the abaxial surface; inflorescences with 2 or occasionally 3 secondary axes at the basal node.
56a. Floral bracts ovate to elliptic, 3–5 mm long; calyx limbs truncate, 1.5–2 mm long ..... *N. fernandezii*
56b. Floral bracts suborbicular, ovate, lanceolate, or triangular to narrowly so, 0.1–2 mm long; calyx limbs lobed, 0.1–2 mm long.
57a. Leaves usually with intersecondary veins not visible on the abaxial surface; floral bracts reduced, hardly evident; flowers borne in umbelliform or racemiform cymules of 3 to 10 ..... *N. uliginosa*
57b. Leaves with intersecondary veins visible on the abaxial surface; floral bracts developed, evident; flowers borne singly or in glomerules or dichasial cymules of 2 to 3.
58a. Leaves 4–8 cm wide; floral bracts 2–4 mm long; pyrenes at maturity dorsiventrally flattened ..... *N. patria*
58b. Leaves 9–17 cm wide; floral bracts 0.5–1.5 mm long; pyrenes at maturity triangular to umbonate in cross section ..... *N. steyermarkiana*
53b. Mature fruits white (color unknown in *N. perparva*).
59a. Flowers grouped in glomerules of 4+, the glomerules borne at the ends of the inflorescence axes and enclosed by lanceolate to ovate bracts that are longer than the hypanthium plus calyx limb.
60a. Leaves 4–12.5 cm wide, with secondary veins 9 to 16 pairs, eucamptodromous or weakly brochidodromous in apical part of blade ..... *N. aggregata*
60b. Leaves 7–24 cm wide, with secondary veins 16 to 30 pairs, markedly brochidodromous throughout.
61a. Peduncles 7–20 cm long; calyx limbs 0.8–1.5 mm long, very shallowly lobed; corolla lobes abaxially smooth ..... *N. submarginalis*
61b. Peduncles 4–8.5 cm long; calyx limbs 1.2–2 mm long, lobed for ¼–¾ their length; corolla lobes abaxially with conical appendages.
62a. Secondary leaf veins 16 to 22 pairs; western Panama ..... *N. nepokroeffiae*
62b. Secondary leaf veins 26 to 30 pairs; eastern Panama ..... *N. perparva*
59b. Flowers borne singly or in glomerules of 2 to 3, the flowers or glomerules distributed along spiciform inflorescence axes and subtended but not enclosed by triangular bracts that are usually shorter than the hypanthium plus calyx limb.

63a. Peduncles in fruit 7–20 cm long; floral bracts absent or very reduced and hardly evident; Venezuela ..... *N. terepaimensis*

63b. Peduncles in fruit 0.8–22 cm long; floral bracts developed and evident; Costa Rica to Bolivia.

64a. Mature pyrenes abaxially with 3 longitudinal ridges, these equally spaced and similar ..... *N. anomothyrsa*

64b. Mature pyrenes with 1 or 2 closely set central longitudinal ridges, sometimes also with 1 equally spaced lateral ridge but these less well developed.

65a. Leaves glabrous or puberulous adaxially, pilosulous to glabrescent abaxially; peduncles 7–22 cm long; Costa Rica to Peru ..... *N. capacifolia*

65b. Leaves pilose or villosulous on both surfaces; peduncles 0.8–5.5 cm long; southern Ecuador to Peru ..... *N. cincinalis*

Notopleura acuta C. M. Taylor, sp. nov. TYPE: Ecuador. Napo: Estación Biológica Jatun Sacha, 450 m, 2 July 1990, *T. Flynn, R. Peckinpaugh & R. Yamakawa 4025* (holotype, MO-4312901; isotypes, PTBG-10899, QCNE not seen). Figure 1A–C.

Haec species a congeneris foliis apice acutis nonnunquam acuminatis, inflorescentia capitata nutante, stipitibus sub fructu usque ad 5 mm longis accrescentibus atque pyrenis dorsiventraliter complanatis marginibus aliquam incrassatis distinguitur.

Terrestrial herbs or subshrubs to 1 m tall, rather succulent, apparently unbranched; stems glabrous or villosulous to pilosulous, becoming glabrescent with age. *Leaf blades* elliptic to oblanceolate, 9.5–17 × 3.5–5 cm, at apex acute to sometimes acuminate, at base acute to cuneate, drying papyraceous to membranaceous, glabrous or pilosulous to villosulous usually becoming glabrescent with age, abaxially sometimes paler; secondary veins 7 to 10 pairs, extending nearly to margins; petioles 1.5–3 cm long, glabrous or pilosulous to villosulous, becoming glabrescent; stipules with sheath 0.5–1.5 mm long, broadly triangular to rounded, entire, glabrous or villosulous, becoming glabrescent, with interpetiolar appendage conical, to 2 mm long, caducous or occasionally persistent. *Inflorescences* pseudoaxillary, pilosulous to villosulous, capitate, nodding, with peduncle 2–5 mm long, head hemispherical to depressed-globose, 5–8 mm diam.; outermost bracts triangular to lanceolate, 4–8 mm long, acute, entire, floral bracts 3–5 mm long; *flowers* sessile, not seen. *Infructescences* subcapitate to contracted-cymose, with peduncles to 15 mm long; *fruits* ellipsoid, 5–6 × 4–5 mm, red becoming black at maturity, with stipes to 5 mm long, with persistent calyx limbs 1–1.5 mm long, lobed for ca. ½ their length, the lobes triangular to narrowly so; pyrenes dorsiventrally flattened, dorsal surface smooth except for 1 low, sharp, central, longitudinal ridge and thickened margins.

Habitat, distribution, and phenology. Ecuador to northern Peru, in wet forest at 140–450 m; collected in flower January, June, and July, in fruit January, May–August.

This species is distinguished by its leaves that are acute to acuminate at the apex, its capitate nodding inflorescences, its fruiting stipes up to 5 mm long, and its dorsiventrally flattened pyrenes that have one sharp, central, longitudinal ridge on the abaxial surface and the margins somewhat thickened. This species has been confused with *Notopleura plagiantha* (Standl.) C. M. Taylor; *N. plagiantha* differs in its fruits that remain subsessile or sessile at maturity and its calyx limbs ca. 2 mm long with linear lobes. The specific epithet refers to the acutely angled leaf apices, which are uncommon in *Notopleura* and a distinguishing character for this species.

Paratypes. ECUADOR. **Napo:** Estación Biológica Jatun Sacha, Río Napo, 8 km al E de Misahuallí, 1°04′S, 77°36′W, *Cerón 1650* (MO), *Gentry et al. 60261* (MO), *Miller et al. 2175* (MO). **Pastaza:** cantón Puyo, Pambayucua, Río Lliquino, 1°29′S, 77°22′W, *Palacios 10155* (MO, QCNE); cantón Pastaza, Villano-Pandanuque, junto al Río Villano, 1°30′S, 77°27′W, *Palacios 10338* (MO, QCNE). PERU. **Loreto:** provincia Maynas, distrito Iquitos, carretera del Varillal, trocha del puesto "El Virosal", cerca al vivero forestal, *Rimachi 6857* (MO).

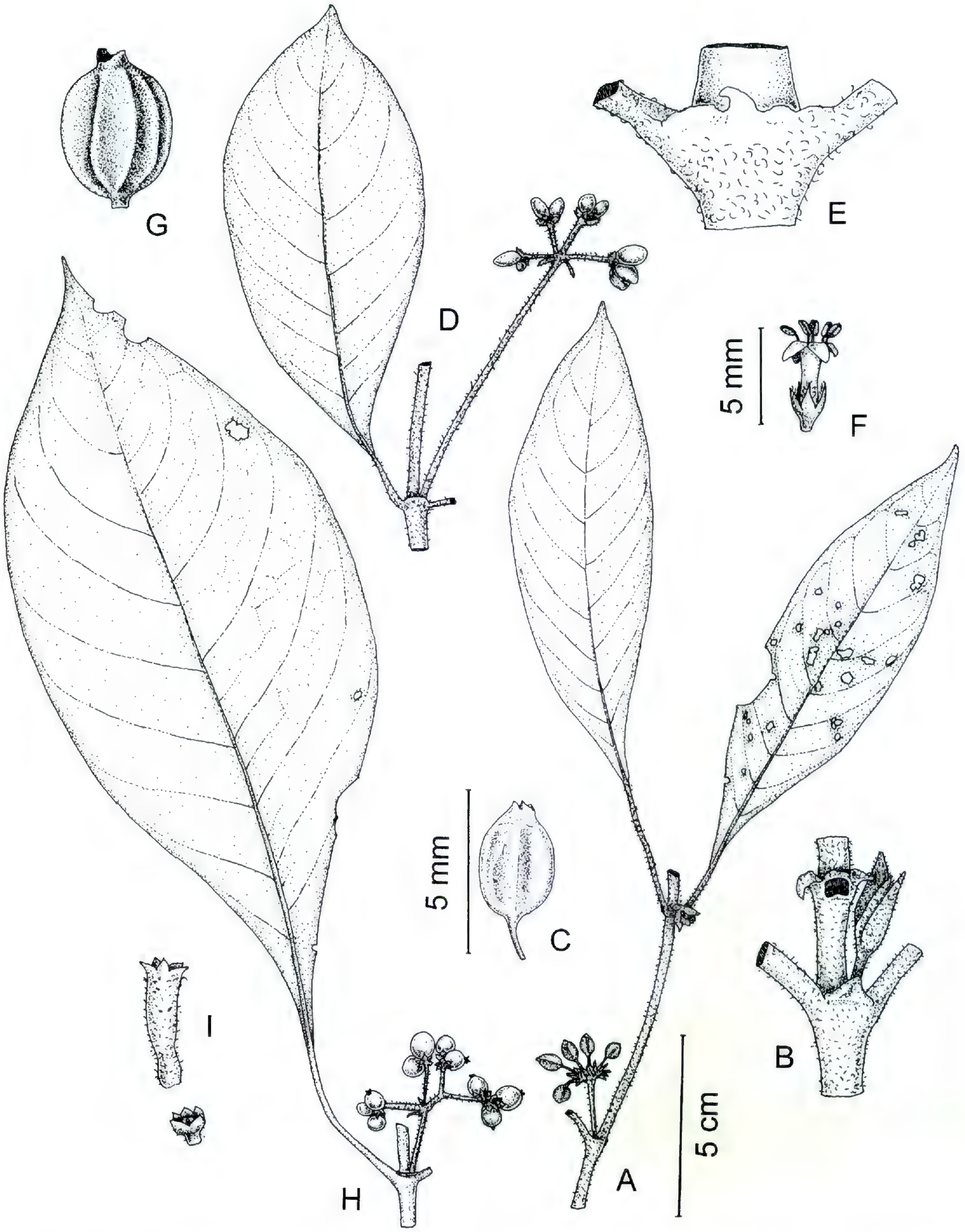


Figure 1. A–C, *Notopleura acuta* C. M. Taylor. —A. Portion of branch with young inflorescence and infructescence. —B. Stem portion showing stipules. —C. Dry immature fruit. D–G, *Notopleura aequatoriana* C. M. Taylor. —D. Portion of branch with infructescence. —E. Stipule. —F. Flower. —G. Dried fruit. H, I, *Notopleura congesta* C. M. Taylor. —H. Portion of branch with infructescence. —I. Flower, partially dissected. A, D, H to 5-cm scale; E, F, G, I to same scale; B, C to same scale. A, C based on *Flynn et al. 4025*; B based on *Miller et al. 2175*; D, G based on *Cerón & Hurtado 3840*; E, F based on *Hurtado 2687*; H based on *Cerón 2291*; I based on *Foster 8586*.

Notopleura aequatoriana C. M. Taylor, sp. nov. TYPE: Ecuador. Napo: cantón Archidona, carretera Hollín–Loreto, entre Avila y Loreto, Huiruna, comunidad Quechua, 00°43′S, 77°19′W, 450 m, 24 Nov. 1989, *C. E. Cerón 7745* (holotype, MO-4247070). Figure 1D–G.

Haec species a congeneris statura humili, foliis sat parvis plerumque abaxialiter pilosulis villosulisve, panicula ramosa aliquam brevi, stipularum vaginis interpetiolaribus late triangularibus ad truncatis atque fructuum rubrorum aurantiacorumve pyrenis dorsiventraliter complanatis crista longitudinali in pagina dorsali et marginibus incrassatis munitis distinguitur.

Terrestrial herbs or subshrubs to 35 cm tall, unbranched; stems pilosulous or villosulous, usually becoming glabrescent with age. *Leaf blades* elliptic to obovate, 8–16 × 3–8 cm, at apex acute, at base cuneate to obtuse, drying papyraceous, adaxially glabrous, abaxially moderately to densely pilosulous or villosulous and apparently paler; secondary veins 7 to 11 pairs, extending to near margins; petioles 1–3 cm long, pilosulous or villosulous becoming glabrescent; stipules with sheath 1–2.5 mm long, truncate to broadly triangular, entire, pilosulous or villosulous becoming glabrescent, with interpetiolar appendage conical, to 1 mm long. *Inflorescences* pseudoaxillary, pilosulous or villosulous, branched, with peduncle 3.5–10 cm long, flowering portion pyramidal to rounded, 1–3 × 1.5–4 cm, with developed secondary axes produced at 1 to 2 nodes, with 3 or sometimes 2 secondary axes produced at basalmost node; bracts triangular, acute, entire, those subtending secondary axes 2–7 mm long, floral bracts 1.2–3 mm long; *flowers* sessile in glomerules of 5 to 15, with hypanthium pilosulous, ca. 0.5 mm long; calyx limb pilosulous, 0.5–2 mm long, 5-lobed for ½–¾ its length, lobes narrowly triangular; corolla slenderly funnelform, externally and internally glabrous, tube ca. 4 mm long, lobes 5, triangular, ca. 1 mm long, abaxially smooth; stamens and stigmas not seen. *Infructescences* similar to inflorescences; *fruits* ellipsoid, 6–8 × 4.5–5.5 mm, red to orange, with stipes to 1.5 mm long; pyrenes 2, dorsiventrally flattened, dorsal surface smooth with 1 low, sharp, central, longitudinal ridge and thickened margins.

Habitat, distribution, and phenology. Central eastern Ecuador, in wet forest at 200–620 m; collected in flower in November, in fruit in February–May and November.

This species is distinguished by its low stature, its relatively small leaves that are usually pilosulous or villosulous on the abaxial surface, its stipules with sheaths broadly triangular to truncate, its branched but rather short panicles, its red to orange fruits, and its pyrenes that are dorsiventrally flattened with a longitudinal ridge on the abaxial surface and thickened margins. The specific epithet refers to the geographic range of this species, which is apparently restricted to Ecuador. The fruits are described as red to orange on all collections studied, but they may ultimately become black as in several other species; such ripe black fruits are rarely collected. *Notopleura aequatoriana* is similar to *N. leucantha* (K. Krause) C. M. Taylor; *N. leucantha* differs in its capitate inflorescences that are subsessile or borne on peduncles up to 1 cm long.

Paratypes. ECUADOR. **Napo:** Parque Nacional Yasuní, pozo petrolero Daimi 2, 00°55′S, 76°11′W, *Cerón & Hurtado 3840* (MO); cantón Archidona, área al sureste del Volcán Sumaco, carretera Hollín–Loreto, Km 65, Huaticocha, 00°45′S, 77°28′W, *Hurtado 2367* (MO); cantón Archidona, Huiruno, al pie del Volcán Sumaco, carretera Hollín–Loreto, 5 km al SO de Loreto, 00°43′S, 77°20′W, *Hurtado 2687* (MO); cantón Orellana, carretera y oleoducto de Maxus en construcción, Km 19, 00°33′S, 76°31′W, *Hurtado 3052* (MO). **Sucumbíos:** cantón Lago Agrio, Dureno, comunidad indígena Cofán, 00°02′S, 76°42′W, *Cerón 217* (MO); along Río Aguarico, 3–5 mi. upstream from Santa Cecilia, *Duke 16062* (MO); Santa Cecilia, near W end of airplane runway, *MacBryde & Dwyer 1287* (MO).

Notopleura aggregata (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria aggregata* Standl., Contr. U.S. Natl. Herb. 18: 128. 1916. TYPE: Panama. Chiriquí: between Alto de las Palmas y Cerro de la Horqueta, 2100–2268 m, 10 Mar. 1911, *H. Pittier 3264* (holotype, US-677655).

Costa Rica to northwestern Colombia, common in Central America but infrequently collected in Colombia.

Notopleura agostinii (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria agostinii* Steyerm., Mem. New York Bot. Gard. 23: 551. 1972. TYPE: Venezuela. Aragua: on Pico Periquito, trail above Pittier monument to summit, Parque Nacional "H. Pittier," 1250–1600 m, 4 Sep. 1960, *J. A. Steyermark & G. Agostini 8* (holotype, NY not seen).

Venezuela.

Notopleura albens C. M. Taylor, sp. nov. TYPE: Ecuador. Carchi: mountains E and NE, along slope of mountain ENE of Rafael Quindí's mountain finca and above Río Verde including top of mountain, 00°52′N, 78°07′W, 1870–2400 m, 3 Dec. 1987, *W. S. Hoover 2260* (holotype, MO-4388879). Figure 2A–D.

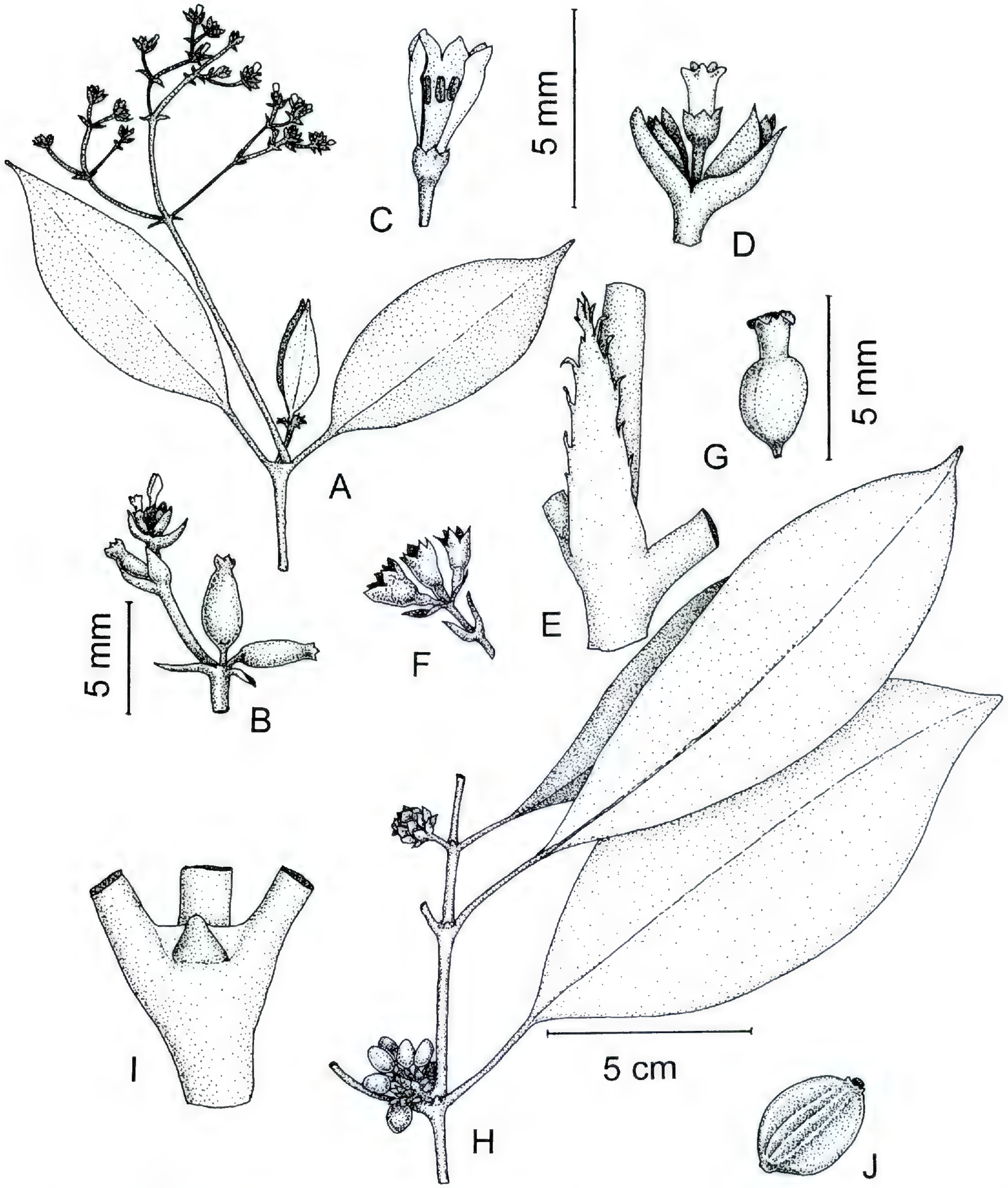


Figure 2. A–D, *Notopleura albens* C. M. Taylor, based on *Hoover 2260*. —A. Branch with inflorescence. —B. Portion of inflorescence with young fruits. —C. Flower, partially dissected. —D. Portion of inflorescence with young flower bud. E–G, *Notopleura callejasii* C. M. Taylor, based on *Callejas et al. 10733*. —E. Stipule. —F. Cymule from inflorescence, with flower after corolla has fallen off. —G. Young fruit. H–J, *Notopleura iridescens* C. M. Taylor, based on *Foster 9231*. —H. Portion of branch with inflorescence and infructescence. —I. Stipule. —J. Dried fruit. A, H to same scale; C, D to same scale; E, F, G, I, J to same scale.

Haec species a congeneris stipularum laminis interpetiolaribus expansis fimbriatis, lamina foliari subtus albente sine nerviis secundariis manifestis, inflorescentiae axibus patentibus valde dichasialibus atque pyrenis sat angustis ca. 4 × 2 mm distinguitur.

Terrestrial herbs to 1 m tall, sometimes creeping, unbranched, with vegetative portions glabrous. *Leaf blades* elliptic, 5.5–11 × 1.8–5.5 cm, at apex acute to slightly acuminate, at base acute to cuneate, dry-

ing subcoriaceous to chartaceous, abaxially whitened; secondary veins not evident; petioles 1–3 cm long; stipules with sheath reduced, interpetiolar appendage laminar, triangular to ligulate, 2–4 mm long, caducous, deeply fimbriate. *Inflorescences* pseudoaxillary, glabrous to puberulous, paniculate, with peduncle 5.5–9 cm long, flowering portion pyramidal to roumded, 5–6 × 4–10 cm, with developed secondary axes produced at 2 to 3 nodes, these 2 per node, these and minor axes widely spreading, strongly dichasial; bracts narrowly triangular, acute, entire or ciliolate, those subtending secondary axes 5–7 mm long, floral bracts 2–3 mm long; *flowers* subsessile, with hypanthium glabrous, ca. 1 mm long; calyx limb glabrous, ca. 0.8 mm long, shallowly 5-lobed, lobes broadly triangular; corolla in bud infundibuliform, white, externally and internally glabrous, tube ca. 3 mm long, lobes ca. 1 mm long, triangular, abaxially with conical appendages to 0.3 mm long; stamens inserted just above middle of corolla tube, anthers ca. 0.8 mm long, positioned at mouth of tube; stigmas ca. 0.8 mm long, positioned just below anthers. *Infructescences* similar to inflorescences; *fruits* narrowly ellipsoid to lanceoloid, ca. 4 × 2 mm, mature color unknown; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Northwestern Ecuador, in wet forest at 1350–2400 m; collected in flower in December, in fruit November–January.

This species is distinguished by its stipules with expanded, fimbriate, interpetiolar laminas 2–4 mm long, its leaves whitened on the abaxial surface and with the secondary veins not visible, its inflorescences with the axes strongly dichasial and spreading, and its relatively narrow pyrenes ca. 4 × 2 mm. This species is similar to *Notopleura callejasii* C. M. Taylor; *N. callejasii* differs in its longer calyx limbs, 3–4.5 mm long. The specific epithet refers to the whitened undersides of the leaves.

Paratypes. ECUADOR. **Carchi:** trail from Rafael Quindí's mountain finca above Río Verde, 00°52′N, 78°08′W, *Hoover & Wormley 1794* (MO); above gorge in river draining medium Golondrinas, 00°52′N, 78°17′W, *Hoover 2256* (MO); trail to Pailon encampment, Gualpí Chico area of Awá Reserve, 00°58′N, 78°16′W, *Hoover et al. 3626* (MO).

Notopleura aneurophylla (Standl.) C. M. Taylor, comb. nov. Basionym: *Cephaelis aneurophylla* Standl., Publ. Field Columbian Mus., Bot. Ser. 8: 63. 1930. *Psychotria aneurophylla* (Standl.) Steyerm., Mem. New York Bot. Gard. 23: 546. 1972. TYPE: Venezuela. Aragua: between Maracay and Choroni, 750 m, 12 Dec. 1856, *A. Fendler 1979* (holotype, F).

Venezuela.

Notopleura aneurophylloides (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria aneurophylloides* Steyerm., Mem. New York Bot. Gard. 23: 547. 1972. TYPE: Venezuela. Yaracuy: en la fila, Cerro La Chapa, norte de Nirgua, 1200–1360 m, 11 Mar. 1967, *J. A. Steyermark & G. Bunting 97655* (holotype, VEN not seen).

Venezuela.

Notopleura angustissima (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria macrophylla* var. *angustissima* Standl., Publ. Field Mus. Nat. Hist., Bot. Ser. 22: 203. 1940. TYPE: Panama. Darién: Caná–Cuasi Trail, N slope, 5500 ft., 16 Mar. 1940, *M. E. Terry & R. A. Terry 1597* (holotype, F).

Eastern Panama and presumably adjacent Colombia. This species has been included by recent authors (e.g., Steyermark, 1972) in *Notopleura macrophylla*, but with more material available now *N. angustissima* can be seen to have red fruits and dorsally smooth pyrenes that are hemispherical in cross section, and appears more closely related to *N. siggersiana* (Standl.) C. M. Taylor and several other species with these same fruit characters.

Notopleura anomothyrsa (K. Schum. & Donn. Sm.) C. M. Taylor, comb. nov. Basionym: *Psychotria anomothyrsa* K. Schum. & Donn. Sm., Bot. Gaz. (Crawfordsville) 35: 3. 1903. *Psychotria macrophylla* subsp. *anomothyrsa* (K. Schum. & Donn. Sm.) Steyerm., Mem. New York Bot. Gard. 23: 563. 1972. TYPE: Guatemala. Alta Verapaz: Cubilquitz, 350 m, Dec. 1900, *von Tuerckheim 7909* (holotype, US-943471 not seen, microfiche; isotypes, GH not seen, MO (s.n.), US not seen).

Central Mexico to northern Peru. This name has been included by recent authors (e.g., Steyermark, 1972) in *Notopleura macrophylla*, but closer study shows that this name applies to a distinct species; see the discussion under *N. macrophylla*, below.

Notopleura araguensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria araguensis* Steyerm., Mem. New York Bot. Gard. 23: 551. 1972. TYPE: Venezuela. Aragua: selvas del Río Grande, Parque Nacional, 14 Sep. 1946, *T. Lasser 2047* (holotype, VEN not seen).

Venezuela.

Notopleura callejasii C. M. Taylor, sp. nov. TYPE: Colombia. Antioquia: municipio Valdivia, vereda San Fermín, sítio "Alto de Ventanas" (municipio Yarumal), en la vía que conduce al municipio de Briceño, 2 km NE de la vía Medellín–Valdivia, 7°05′N, 75°32′W, 1900–2100 m, 18 Sep. 1992, *R. Callejas, C. Taylor, F. J. Roldan & A. Gómez 10733* (holotype, HUA-83354). Figure 2E–G.

Haec species a *Notopleura albens* C. M. Taylor limbo calycino 3–4.5 mm longo distinguitur.

Hemiepiphytic, suffrutescent herbs, unbranched, with vegetative portions glabrous. *Leaf blades* elliptic to elliptic-oblong, 8.5–14 × 4–6.5 cm, at apex acuminate, at base cuneate to obtuse, drying chartaceous; secondary veins not or hardly evident; petioles 1–2 cm long; stipules with sheath reduced, interpetiolar appendage laminar, lanceolate to triangular, 12–14 mm long, caducous, deeply fimbriate. *Inflorescences* pseudoaxillary, glabrous to puberulous, paniculate, with peduncle ca. 7 cm long, branched portion pyramidal to rounded, ca. 6 × 11 cm, with developed secondary axes produced at 3 nodes, these 2 per node; bracts narrowly triangular to ligulate, acute to acuminate, entire or ciliolate, those subtending secondary axes 5–17 mm long, floral bracts 1–3 mm long; *flowers* subsessile in cymules of 3(to 5), with hypanthium glabrous, ca. 1 mm long; calyx limb glabrous, 3–4.5 mm long, 5-lobed for ca. ¼ its length, lobes narrowly triangular, acute to acuminate; corolla, stamens, and stigmas not seen. *Infructescences* similar to inflorescences; *fruits* ellipsoid, 4–8 × 4–6 mm, bright red; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Known only from the type collection, in wet montane forest at 1900–2100 m in northwestern Colombia; collected in immature fruit in September.

This species is distinguished by its leaves with the secondary veins not or hardly visible, its stipules with expanded, strongly fimbriate laminar appendages, its inflorescences with spreading axes and well-developed bracts, and its calyx limbs ca. 4.5 mm long. It is similar to *Notopleura albens* of northwestern Ecuador; *N. albens* differs in its calyx limb only about 0.8 mm long. The name honors Ricardo Callejas, professor at the University of Antioquia and a botanist whose work has greatly advanced our knowledge of the flora of the Neotropics, and of Antioquia Department in particular.

Notopleura camponutans (Dwyer & M. V. Hayden) C. M. Taylor, comb. nov. Basionym: *Cephaelis camponutans* Dwyer & M. V. Hayden, Ann. Missouri Bot. Gard. 55: 35. 1968. *Psychotria camponutans* (Dwyer & M. V. Hayden) Hammel, Selbyana 12: 139. 1991. TYPE: Panama. Panamá: Cerro Jefe, *J. D. Dwyer & G. Gauger 7346* (holotype, MO not seen; isotype, F).

Cephaelis gaugeri Dwyer, Ann. Missouri Bot. Gard. 67: 71. 1980. Syn. nov. TYPE: Panama. Panamá: Cerro Jefe, near junction of roads to Cerro Jefe and Altos de Pacora, 18 Dec. 1974, *S. Mori & J. Kallunki 3779* (holotype, MO-224077).

Costa Rica to central Panama.

Notopleura capacifolia (Dwyer) C. M. Taylor, comb. nov. Basionym: *Psychotria capacifolia* Dwyer, Ann. Missouri Bot. Gard. 67: 353. 1980. TYPE: Panama. Bocas del Toro: Bonyik, 500–1200 ft., 13 Apr. 1968, *J. H. Kirkbride, Jr. & J. Duke 601* (holotype, MO-2623364; isotype, MO-2263363).

Psychotria dosbocensis Dwyer, Ann. Missouri Bot. Gard. 67: 370. 1980. Syn. nov. TYPE: Panama. Veraguas: valley of Río Dos Bocas on road between Alto Piedra (above Santa Fe) and Calovébora, 350–400 m, 29 Aug. 1974, *T. B. Croat 27427* (holotype, MO-2216296).

Costa Rica to Peru.

Notopleura chapensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria chapensis* Steyerm., Mem. New York Bot. Gard. 23: 552. 1972. TYPE: Venezuela. Yaracuy: al norte de Nirgua, Cerro La Chapa, 1200–1400 m, 9–10 Nov. 1967, *J. A. Steyermark, G. Bunting & G. Wessels-Boer 100217* (holotype, VEN not seen).

Venezuela.

Notopleura cincinalis C. M. Taylor, sp. nov. TYPE: Peru. Amazonas: provincia Condorconqui, distrito El Cenepa, región Nororiental del Marañón, Río Cenepa, comunidad Tutino, 04°33′S, 78°10′W, 450 m, 22 Nov. 1993, *R. Vásquez, C. Díaz, J. Mostacero, F. Mejía & J. Ampam 18503* (holotype, MO-5206807). Figure 3A–C.

Haec species a congeneris foliis pubescentia pilosula villosulave tectis, inflorescentia axibus secundariis cincinnalibus praedita pedunculo sat brevi insidente atque fructuum maturitate alborum pyrenis dorsiventraliter complanatis crista longitudinali in pagina dorsali munitis distinguitur.

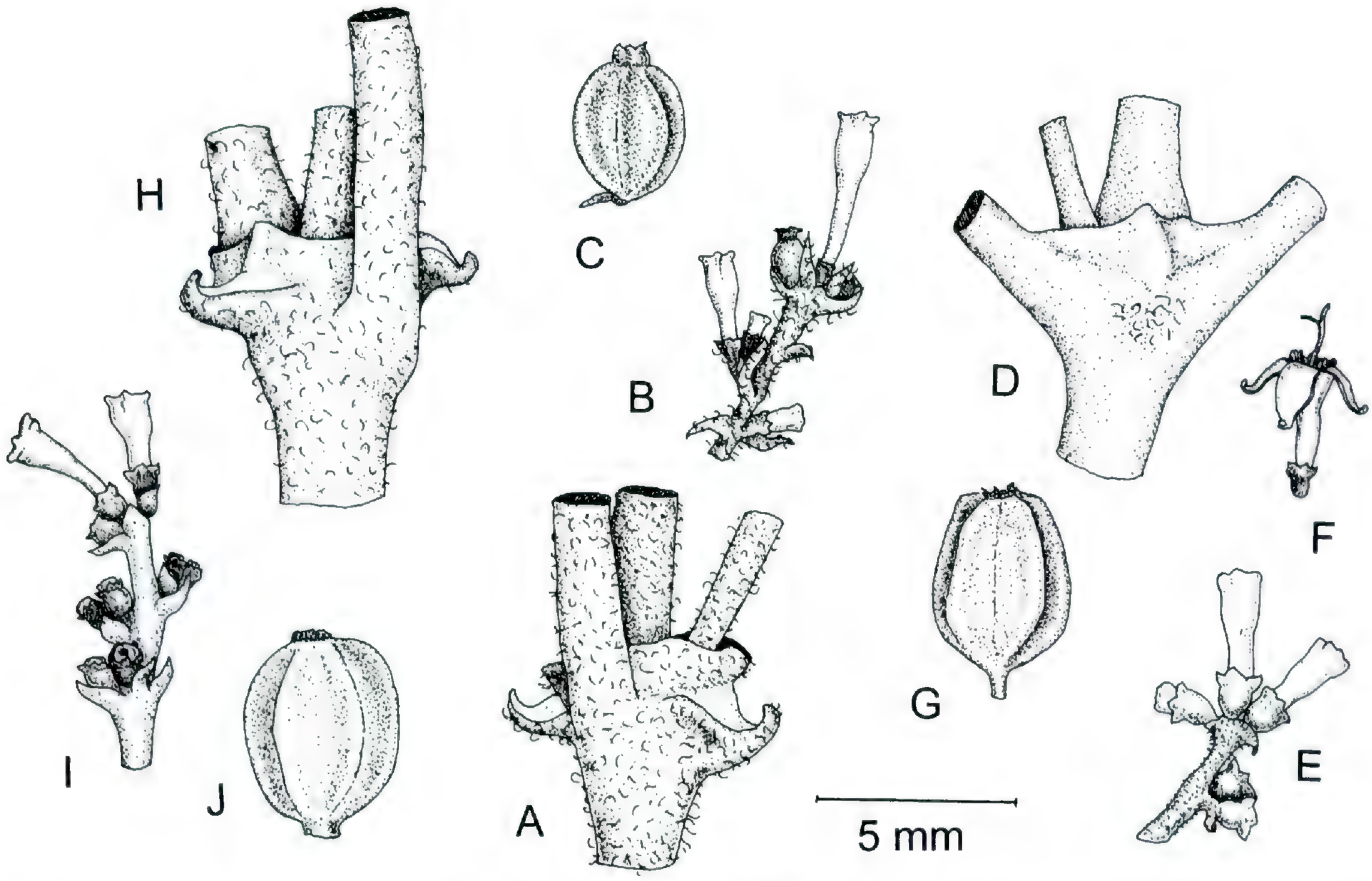


Figure 3. A–C, *Notopleura cincinalis* C. M. Taylor. —A. Portion of stem with stipule. —B. Portion of inflorescence with flower buds and young fruits. —C. Dried fruit. D–G, *Notopleura steyermarkiana* C. M. Taylor. —D. Portion of stem with stipule. —E. Portion of inflorescence with flower buds. —F. Flower. —G Dried fruit. H–J, *Notopleura triaxillaris* C. M. Taylor. —H. Portion of stem with stipule. —I. Portion of inflorescence with flower buds. —J. Dried fruit. All to same scale. A based on *Vásquez et al. 18503*; B based on *Kayap 521*; C based on *Ancuash 64*; D, F based on *Kessler 2589*; E, G based on *Liesner & González 9817*; H, I based on *Foster & Smith 9426*; J based on *Schunke Vigo 10158*.

Terrestrial herbs or shrubs to 1 m tall, apparently unbranched; stems pilosulous or villosulous usually becoming glabrescent. *Leaf blades* elliptic to broadly so, 20–30 × 9–14.5 cm, at apex shortly acuminate, at base cuneate to rounded, drying membranaceous to papyraceous, on both surfaces sparsely to moderately pilose or villosulous but more densely so on principal veins, abaxially apparently paler; secondary veins 13 to 17 pairs, extending to near margins or usually uniting in a distinct submarginal vein especially in distal part of blade; petioles 2.5–7.5 cm long, villosulous or pilosulous, usually becoming glabrescent; stipules with sheath 3–5 mm long, broadly rounded to broadly triangular, entire or ciliolate, pilosulous or villosulous, subcoriaceous, deciduous, with interpetiolar appendage to 5 mm long, conical, fimbriate, glandular, caducous. *Inflorescences* pseudoaxillary, villosulous or pilosulous, paniculate, with peduncle 0.8–5.5 cm long, branched portion pyramidal, 2–6 × 1.5–6.5 cm, with well-developed secondary axes produced at 3 to 4 nodes, these 2 per node, cincinnoid, with flowers or glomerules separated and apparently alternate along them; bracts triangular, acute, entire, those subtending secondary axes 3–8 mm long, floral bracts 1–2 mm long; *flowers* sessile, borne singly or in glomerules of 2 to 3, with hypanthium villosulous or pilosulous, ca. 0.5 mm long, turbinate; calyx limb pilosulous or villosulous, 0.5–0.8 mm long, shallowly 5-lobed, lobes triangular; corolla in bud slenderly infundibuliform, white, externally glabrous except pilosulous or puberulous on lobes, internally glabrous except pilosulous just above middle of tube, tube to 5.5 mm long, lobes to 1 mm long, triangular, a little thickened abaxially near apex; stamens inserted just below mouth of corolla tube, anthers ca. 1 mm long, probably exserted; stigmas ca. 1 mm long, position at anthesis unknown. *Infructescences* similar to inflorescences; *fruits* ellipsoid, 4.5–5 × 4 mm, white; pyrenes 2, dorsiventrally flattened, dorsal surface flat with 1 low, sharp, central, longitudinal ridge and margins thickened.

Habitat, distribution, and phenology. Southern Ecuador to northern Peru, in wet forest at 250–1000 m; collected in flower in March, in fruit in March, July, October, and November.

This species is distinguished by its pilosulous to villosulous leaves, its inflorescences with relatively short peduncles and the secondary axes cincinnoid, its fruits that are white at maturity, and its pyrenes that are dorsiventrally flattened with a longitudinal central ridge on the dorsal surface and thickened margins. The specific epithet refers to the form of the secondary inflorescence axes.

Paratypes. ECUADOR. **Zamora–Chinchipe:** cantón Nangaritza, Río Nangaritza, Pachicutza, camino al hito de Pachicutza, 4°07′S, 78°37′W, *Palacios et al. 8213* (MO). PERU. **Amazonas:** alrededor de la comunidad Kusu, Río Numpatkin, *Ancuash 64* (MO), *Kayap 521* (MO); al lado de Cenepa, *Kayap 1258* (MO).

Notopleura congesta C. M. Taylor, sp. nov. TYPE: Ecuador. Napo: cantón Archidona, faldas al S del volcán Sumaco, carretera Hollín–Loreto, Km 31, comuna Challua Yacu, 00°43′S, 77°36′W, 1200 m, 8–17 Jan. 1989, *A. Alvarado 235* (holotype, MO-3905983). Figure 1H, I.

Haec species a congeneris surculis pubescentia villosula pilosulave tectis, stipulis 0.5–2 mm longis, inflorescentia congesta flores in (1 vel) 3–5 glomerulos et bracteas 2–8 mm longas gerente, lobulis corollinis exappendiculatis atque fructuum maturitate rubrorum aurantiacorumve pyrenis in sectione transversali semicircularibus in pagina dorsali laevibus distinguitur.

Terrestrial herbs or shrubs to 1.8 m tall, apparently unbranched; stems densely villosulous to pilosulous often becoming glabrescent. *Leaf blades* elliptic to obovate, 14–29 × 4–10 cm, at apex acute to acuminate, at base acute to attenuate, drying membranaceous to papyraceous, adaxially glabrous to sparsely puberulous, abaxially pale and moderately to densely villosulous or pilosulous; secondary veins 7 to 9 pairs, extending to near margins; petioles 1–4 cm long, pilosulous or villosulous; stipules with sheath 0.5–2 mm long, truncate to broadly rounded, densely pilosulous or villosulous, generally persistent, without interpetiolar appendage. *Inflorescences* pseudoaxillary, pilosulous or villosulous, capitate to paniculate, nodding, with peduncle 3–4 mm long, flowering portion capitate and hemispherical or branched with 3 to 5 capitula or glomerules, 5–8 × 8–10 mm; bracts triangular, acute, the outermost 4–8 mm long, floral bracts 2–4 mm long; *flowers* sessile, in bud with calyx limb shallowly 5-lobed, 1–1.5 mm long, and corolla lobes 5, abaxially with linear appendages ca. 0.3 mm long. *Infructescences* similar to inflorescences except more expanded, with peduncles to 1.5 cm long, fruiting portion to 1.5 × 5 cm, sometimes becoming branched with 3 to 5 capitula or glomerules; *fruits* ellipsoid to subglobose, 5–6 × 4.5–5 mm, at maturity red to orange; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Central eastern Ecuador to central Peru, in wet forest at 250–1200 m; collected in flower bud in January, October, and December, in fruit in February, October, and December.

This species is distinguished by its villosulous or pilosulous young growth, its truncate unappendaged stipule sheaths 0.5–2 mm long, its congested inflorescences with the flowers borne in one or usually three to five glomerules, its bracts 2–8 mm long, its red to orange fruits, and its pyrenes that are hemispherical in cross section and smooth on the dorsal surface. The specific epithet refers to the glomerulate to capitate inflorescence arrangement. This new species is similar to *Notopleura siggersiana* (Standl.) C. M. Taylor of Central America; *N. siggersiana* differs in its truncate to broadly rounded stipule sheaths 3–5 mm long and its leaves 6–11 cm wide. Vegetatively *N. congesta* is also similar to *N. acuta*; *N. acuta* differs in its always capitate inflorescences, its black fruits borne on stipes to 5 mm long, and its dorsiventrally flattened and longitudinally ridged pyrenes.

Paratypes. ECUADOR. **Napo:** cantón Archidona, faldas al S del volcán Sumaco, carretera Hollín–Loreto, Km 31, comuna Challua Yacu, 00°43′S, 77°36′W, *Alvarado 169* (MO); Payamino, Reserva Florística "El Chuncho", 00°30′S, 77°01′W, *Cerón et al. 2291* (MO); along road between Baeza and Lago Agrio, *Croat 49499* (MO); carretera Hollín–Loreto, Km 40–50, 00°43′S, 77°36′W, *Hurtado 870* (MO); en 2 trochas, la una a 300 m del río Challua y la otra a 400 m W, *Hurtado 1230* (MO). **Sucumbíos:** along Río Aguarico, 3–5 mi. upstream from Santa Cecilia, *Duke 16073* (MO). PERU. **Amazonas:** prov. Bagua, 43 km (by road) NE of Chiriaco, *Barbour 4510* (MO). **Pasco:** prov. Oxapampa, Pichis Valley, Santa Rosa de Chivis, Ríos Nochos, 9 km SW of Puerto Bermúdez on new highway, 10°20′S, 74°58′W, *Foster 8586* (MO).

Notopleura cundinamarcana C. M. Taylor, sp. nov. TYPE: Colombia. Cundinamarca: bosque nuboso de San Miguel, 2700 m, 17 dic. 1959, *L. E. Mora 799* (holotype, COL-94247). Figure 4A–C.

Haec species a *Notopleura siggersiana* (Standl.) C. M. Taylor inflorescentia 1–5 glomerulos capitulave gerente, limbo calycino ca. 1.5 mm longo atque tubo corollino 5–7 mm longo distinguitur.

Terrestrial herbs or shrubs to 2 m tall, apparently unbranched; stems glabrous. *Leaf blades* elliptic to somewhat lanceolate, 15–17 × 5.5–8 cm, at apex acuminate, at base cuneate to attenuate, drying papyraceous to chartaceous, adaxially shortly and

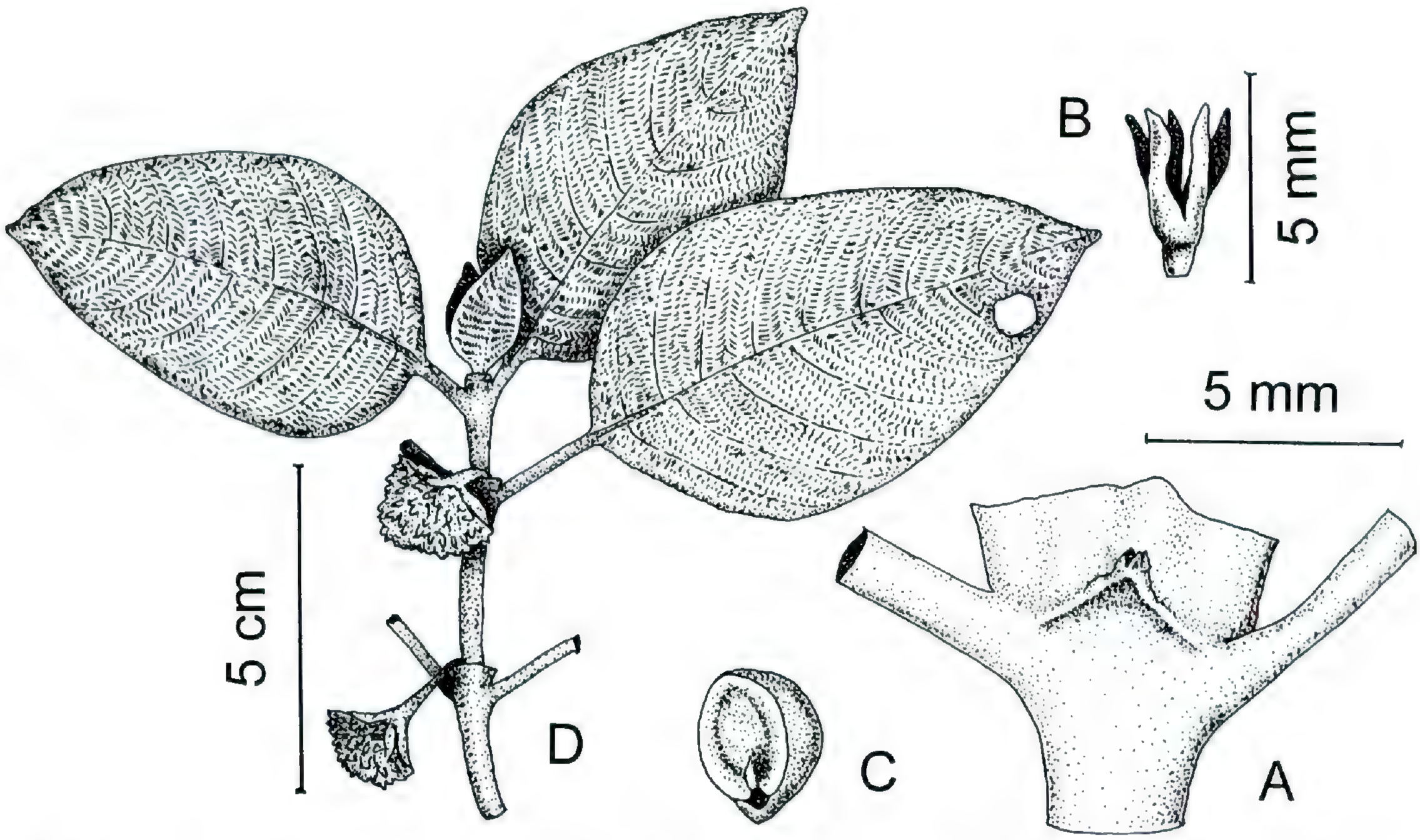


Figure 4. A–C. *Notopleura cundinamarcana* C. M. Taylor, based on *Huertas & Camargo 5168*. —A. Portion of stem with stipule. —B. Calyx. —C. Pyrene, ventral (adaxial) view. —D. *Notopleura lanosa* C. M. Taylor, branch with inflorescence, based on *Silverstone-Sopkin et al. 4968*. A, C to same scale.

stiffly hirsutulous, abaxially hirsutulous to hirsute and usually paler; secondary veins 9 to 12 pairs, usually extending to unite weakly to clearly with margins; petioles 2.5–4 cm long, sparsely hirsutulous to glabrous; stipules with sheath truncate to broadly triangular, 5–7 mm long, membranaceous, entire, glabrous, with interpetiolar appendage 1.5–2 mm long, conical, apparently glandular adaxially and sometimes this glandular portion extending up on sheath immediately distal to appendage. *Inflorescences* pseudoaxillary, glabrous or peduncles hirsutulous, erect to deflexed, capitate to paniculate, with peduncle 1–8.5 cm long, flowering portion pyramidal, 2.5–4.5 × 2.5–6.5 cm, capitate or with 2 to 5 glomerules or capitula, usually with only 1 node, this producing 3 secondary axes, 2 longer and 1 shorter; bracts narrowly triangular to lanceolate or ovate, acute to acuminate, entire or ciliate, those subtending secondary axes 15–25 mm long and united at the base, those in the glomerules or capitula 5–10 mm long; *flowers* sessile, with hypanthium turbinate, ca. 1.5 mm long, glabrous; calyx limb glabrous, 2.5–5 mm long, 5-lobed nearly to base, lobes narrowly triangular, acute; corolla white, slenderly funnelform, glabrous throughout, tube 5–6 mm long, lobes 5, deltoid, ca. 1.5 mm long, abaxially smooth; anthers in apparent short-styled form ca. 1.2 mm long, exserted; stigmas in apparent long-styled form ca. 0.3 mm long, exserted. *Infructescences* similar to inflorescences; *fruit* ellipsoid to subglobose, ca. 5 mm diam., mature color unknown; pyrenes 2, hemispherical, dorsally smooth.

Habitat, distribution, and phenology. In wet montane and cloud forest at 1900–2870 m in Cundinamarca Department of the Cordillera Oriental of Colombia; collected in flower in June and October, in fruit in June and September.

This species is distinguished by its combination of hirsutulous to hirsute leaves and glabrous stems, its stipules with well-developed sheaths with conical interpetiolar appendages, its inflorescences capitate or with 2 to 5 capitula or glomerules, in the latter case with three unequal secondary axes produced at the basal node, its calyx limbs 2.5–5 mm long, its corolla tubes 5–6 mm long, and its hemispherical pyrenes that are smooth dorsally. *Notopleura cundinamarcana* is similar to *N. siggersiana*; *N. siggersiana* differs in its inflorescences with several orders of branching and more numerous, smaller glomerules of flowers, its calyx limbs 0.5–1 mm long, and its corolla tubes ca. 3 mm long. The specific epithet refers to the region where the type was collected.

Paratypes. COLOMBIA. **Cundinamarca:** municipio de Sibate, vereda Paloquemao, *Huertas & Camargo 5168* (COL); Cerro Quininí, *Idrobo 5360* (COL); vereda Agua

Bonita, alrededores de la casa de la Virgen, Alto de San Miguel, *Orozco & Linares 1757* (COL); San Miguel, *Schneider 665* (COL).

Notopleura decurrens (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria decurrens* Steyerm., Mem. New York Bot. Gard. 23: 549. 1972. TYPE: Venezuela. Yaracuy: arriba de San Felipe, Sierra de Aroa, Cerro Negro, en las cabeceras afluentes del Río Cocorotico, 1100–1250 m, 13 Nov. 1967, *J. A. Steyermark & G. Wessels-Boer 100391* (holotype, VEN not seen).

Venezuela.

Notopleura discolor (Griseb.) C. M. Taylor, comb. nov. Basionym: *Psychotria uliginosa* var. *discolor* Griseb., Fl. Brit. W. Ind. Islands 340. 1861. *Psychotria discolor* (Griseb.) Rolfe, Kew Bull. 1893: 258. 1893. SYNTYPES: Jamaica, Dominica, St. Lucia, Trinidad, not cited (K not seen).

Psychotria caribaea Urb., Symb. Ant. 7: 436. 1913. SYNTYPES: Guadeloupe. In Bains-Jaunes (le long du canal de Montèreau), Matouba (coulée de la Ravine-à-Déjeuner), Capesterre (bois de environs du Grand-Etang), in sylvis humidis Grande Découverte, Trois Rivières, 100–900 m, *Duss 2768* (not seen). Dominica. In sylvis prope Laudat, Jan, *Eggers 1012* (not seen); in Morne Baileybook, Mar., *Ramage s.n.* (not seen). Martinique. In Ravine de la Montagne Pelée, Nov., *Hahn 602* (not seen); in montibus Case-Pilote et Case-Navaire, in sylvis Bambou-gouverneur, prope Fontaine Absalon, in Camp de L'Alma, in sylvis Lorrain, *Duss 601* (not seen).

Jamaica and the Lesser Antilles.

Notopleura dukei (Dwyer) C. M. Taylor, comb. nov. Basionym: *Psychotria dukei* Dwyer, Ann. Missouri Bot. Gard. 67: 371. 1980. TYPE: Panama. Darién: Casi-Cana Trail on Cerro Campamiento, E of Tres Bocas, headwaters of Río Coas, 29 Apr. 1968, *J. H. Kirkbride, Jr. & J. A. Duke 1245* (holotype, MO-216265).

Panama. Dwyer's original circumscription of this species included one paratype specimen (*Mori & Kallunki 3750*, cited as "Mori et al.") that actually represents *Notopleura longissima*.

Notopleura fernandezii (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria fernandezii* Steyerm., Mem. New York Bot. Gard. 23: 554. 1972. TYPE: Venezuela. Yaracuy: Pitiguao, camino a Aroa, 1600 m, Jan. 1950, *B. Trujillo & Fernández 766* (holotype, MAR not seen).

Venezuela.

Notopleura hondurensis C. M. Taylor, sp. nov. TYPE: Honduras. Cortés: ca. 30 km above Río de Cusuco, along trail from new park station building to Cerro Cusuco, ca. 23 km W of San Pedro de Sula, ca. 16 km S of Cuyamel, Parque Nacional Cusuco, 15°30′N, 88°13′W, 1640 m, 19 Mar. 1993, *R. Evans 1468* (holotype, MO-4591067). Figure 5A–C.

Haec species a congeneris inflorescentia infructescenciaque pedunculo sat longo insidentibus, vagina stipulari 1.5–2 mm longa atque pyrenis in sectione transversali semicircularibus in pagina dorsali laevibus distinguitur.

Terrestrial herbs or shrubs to 2 m tall, apparently unbranched; stems glabrous. *Leaf blades* elliptic to somewhat ovate, 15–29 × 6.5–15 cm, at apex acuminate, at base obtuse, drying membranaceous, adaxially glabrous, abaxially densely puberulous to pilosulous quickly becoming glabrescent; secondary veins 8 to 12 pairs, generally extending to unite at least weakly with margins; petioles 3–10 cm long, puberulous quickly becoming glabrescent; stipules with sheath 1.5–2 mm long, truncate to broadly rounded, entire, glabrous, membranaceous, sometimes with conical interpetiolar appendage to ca. 1 mm long. *Inflorescences* pseudoaxillary, puberulous to pilosulous, paniculate, with peduncle 8–23 mm long, branched portion pyramidal, 3–11 × 3–16 cm, with developed secondary axes at 2 to 4 nodes, 2 per node; bracts entire, those subtending secondary axes lanceolate to narrowly triangular, 2–6 mm long, acute, floral bracts deltoid to ovate, 1–2 mm long; *flowers* sessile and subsessile in glomerules or congested cymules of 3 to 7, with hypanthium glabrous, ca. 1 mm long; calyx limb glabrous, 0.8–1 mm long, 5-lobed for ca. ½ its length, lobes deltoid; corolla infundibuliform, white, externally and internally glabrous, tube 5.5–7 mm long, lobes 5, 3–3.5 mm long, triangular, abaxially smooth; stamens inserted just above middle of tube, anthers ca. 2 mm long, held at mouth of tube; stigmas ca. 1 mm long, exserted. *Infructescences* similar to inflorescences except usually more expanded, to 22 cm wide; *fruits* ellipsoid, ca. 5 × 5 mm, mature color unknown; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Central Guatemala to Honduras, in wet forest at 1548–1840 m; collected in flower in February, March, and June, in fruit in May.

This species is distinguished by its relatively long peduncles, its stipule sheaths 1.5–2 mm long,

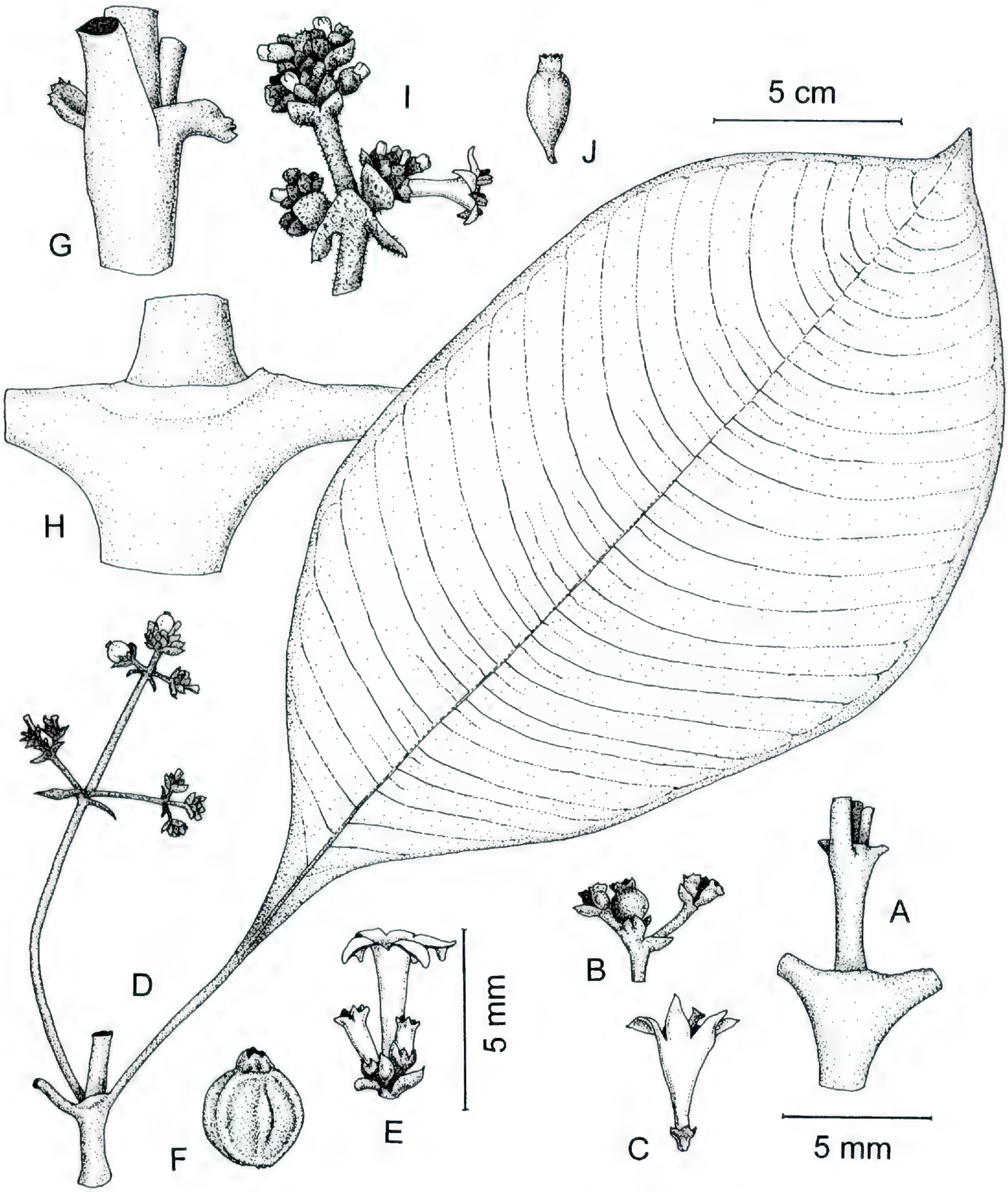


Figure 5. A–C, *Notopleura hondurensis* C. M. Taylor, based on *Evans 1468*. —A. Portion of stem with stipules. —B. Portion of inflorescence with immature fruit. —C. Flower at anthesis. D–F, *Notopleura nepokroeffiae* C. M. Taylor. —D. Portion of branch, showing adaxial leaf surface and inflorescence with young fruits. —E. Portion of inflorescence, with flower buds and flower at anthesis. —F. Dried fruit. D, E based on *Hammel 7413*; F based on *Knapp et al. 2093*. G–J, *Notopleura submarginalis* C. M. Taylor. —G. Stipule from node at stem apex. —H. Portion of stem with stipule fallen, second node down from stem apex. —I. Portion of inflorescence with flower buds and flower at anthesis. —J. Dried fruit. G, H, J based on *Churchill 5775*; I based on *Knapp 4988*. A, B, C, G, H, I, J to same 5-mm scale; E, F to same 5-mm scale.

and its pyrenes that are hemispherical in cross section and smooth on the dorsal surface. This species has been confused previously with *Notopleura macrophylla* and *N. siggersiana*; *N. macrophylla* differs in its pyrenes with 3–5 narrow longitudinal ridges on the dorsal surface, and *N. siggersiana* differs in its stipules with sheaths 3–5 mm long and its pilosulous pubescence on the vegetative parts.

The specific epithet refers to the country where most of the specimens were collected.

Paratypes. GUATEMALA. **Baja Verapaz:** Niño Perdido, Cerro Verde, *Lundell & Contreras 21101* (MO). HONDURAS. **Comayagua:** Cerro Azul Meámbar, on ridge leading to the peak, 10.5 km E of Lago Yojoa, 14°48′N, 87°53′W, *Thomas 618* (EAP, MO). **Copán:** S slopes of Cerro Azul, 12 km NE of Florida, Cerro Azul National Park, 15°06′N, 88°55′W, *Thomas & Mejía 249* (EAP, MO). **Cortés:** a ambos lados del sendero a Cerro Cantiles, 20 km al O de San Pedro Sula, Parque Nacional Cusuco, 15°30′N, 88°14′W, *Mejía 328* (EAP, MO, TEFH). **Yoro:** Río Pijol Valley, 6–7 km S of Nueva Esperanza, 15°12′N, 87°35′W, *Liesner 26571* (MO).

Notopleura humensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria humensis* Steyerm., Mem. New York Bot. Gard. 23: 548. 1972. TYPE: Venezuela. Sucre: entre La Laguna y Roma, NO de Irapa, Cerro de Humo, Peninsula de Paria, 800–1000 m, 5 Mar. 1966, *J. A. Steyermark 95075* (holotype, VEN not seen).

Venezuela.

Notopleura iridescens C. M. Taylor, sp. nov. TYPE: Peru. Huánuco: Pachitea, Codo de Pozuzo, forested alluvial fan floodplain of Río Pozuzo after it emerges from mountains, trail W of settlement to forested lower montane slopes, 9°40′S, 75°28′W, 500–1000 m, 17 Oct. 1982, *R. B. Foster 9231* (holotype, MO-3004162). Fig. 2H–J.

Haec species a *Notopleura plagiantha* (Standl.) C. M. Taylor foliorum nerviis secundariis abaxialiter haud vel vix manifestis, stipulis truncatis tubularibus atque inflorescentia capitata subcapitatave sessili pedunculatave distinguitur.

Terrestrial herbs or shrubs to 0.8 m tall, apparently unbranched; stems glabrous to puberulous. *Leaf blades* elliptic to elliptic-oblong, 10–27 × 4–10 cm, at apex deltoid to acute or a little obtuse, at base cuneate to acute, drying papyraceous, glabrous, abaxially paler; secondary veins not or hardly visible, approximately 10 to 11 pairs; petioles 1–7 cm long, glabrous; stipules with sheath 1–2 mm long, truncate to broadly triangular, entire, glabrous, coriaceous, with thickened midrib sometimes extending to the interpetiolar appendage, this conical, to 1 mm long. *Inflorescences* pseudoaxillary, glabrous, capitate to subcapitate, with peduncle 0–6 mm long, head ovoid to hemispherical, 8–20 mm diam.; outermost bracts lanceolate to ovate, 5–7 mm long, acute, entire, with margins frequently somewhat involute, floral bracts ligulate to oblanceolate, 3–7 mm long, obtuse to rounded; *flowers* sessile, with hypanthium glabrous, ca. 0.5 mm long; calyx limb glabrous, 0.5–0.6 mm long, shallowly 5-lobed, lobes triangular; corolla in bud tubular to somewhat infundibuliform, apparently white, externally glabrous, internally glabrous except for a densely pilosulous ring near middle of tube, tube ca. 5 mm long, lobes 5, ca. 1 mm long, triangular, abaxially smooth; stamens inserted just above middle of tube, anthers ca. 0.8 mm long, held at mouth of tube. *Infructescences* similar to inflorescences or often somewhat more expanded, with peduncle to 5.5 cm long and often becoming thickened distally; *fruits* ellipsoid, 7–8 × 5–6 mm, red, with stipes to 5 mm long; pyrenes 2, dorsiventrally flattened, dorsal surface flat with 1 or 3 low, sharp, longitudinal ridges.

Habitat, distribution, and phenology. Amazonian southern Colombia to northeastern Peru, in wet forest, generally at 115–210 m except to 1400 m in Colombia; collected in flower in January, February, September, and October, in fruit January, May, June–August, and November.

This species is distinguished by its leaves with the secondary veins not or hardly visible on the abaxial leaf surface, its truncate to broadly triangular stipule sheaths, and its pedunculate, capitate to subcapitate inflorescences. It has been confused previously with *Notopleura plagiantha*; *N. plagiantha* differs in its stipules with expanded interpetiolar laminas and its leaves with the secondary veins clearly evident on the abaxial surface. The abaxial leaf surface of *N. iridescens* reportedly has a "silvery-shiny" or "iridescent" surface; the specific epithet refers to this feature.

Paratypes. COLOMBIA. **Putumayo:** municipio Mocoa, corregimiento de San Antonio, vereda Alto Campucana, finca La Mariposa, 1°12′N, 76°38′W, *Betancur et al. 5250* (COL, MO), *Franco et al. 5447* (COL, MO). ECUADOR. **Napo:** behind Hotel Jaguar, 1 hr. down Río Napo by canoe from Misahuallí, *D'Arcy 14028* (MO); cantón Aguarico, Reserva Etnica Huaorani, Maxus Road and pipeline construction project, Kms 60–61, 00°47′S, 76°26′W, *Pitman 255* (MO, QCNE). **Pastaza:** cantón Pastaza, pozo petrolero "Corrientes" de UNOCAL, 01°43′S, 76°49′W, *Gudiño 540* (MO); vía Auca, 115 km al S de Coca, 10 km al S de la frontera Napo-Pastaza, cerca del río Tigüino, carretera de PETRO-CANADA en construcción, 1°15′S, 76°55′W, *Hurtado & Neill 1534* (MO); Río Curaray, costado sur, frente a las lagunas Patoamo, *Neill & Palacios 6810* (MO); Río Curaray, 2 horas río abajo del pueblo Curaray, en la boca del río Namoyacu, 1°24′S, 76°45′W, *Palacios & Neill 610* (MO); cantón Pastaza, pozo petrolero "Ramírez", 20 km al S de la población de Curaray, 1°32′S, 76°51′W, *Zak & Espinoza 5269* (MO). PERU. **Amazonas:** provincia Bagua, distrito Imaza, comunidad de Kampaensa, 4°55′S, 78°19′W, *E. Rodríguez 579* (MO). **Loreto:** provincia Alto Amazonas, Andoas (margen izquierda del Río Pastaza), Campamento OXI,

2°55′S, 76°25′W, *Vásquez & Jaramillo 1920* (MO); provincia Alto Amazonas, Andoas, 2°55′S, 76°25′W, *Vásquez & Jaramillo 4535* (MO); provincia Loreto, carretera Nauta–Iquitos, 4°29′S, 73°35′W, *Vásquez 2225* (MO); provincia Loreto, Nauta, Río Marañón, 4°29′S, 73°35′W, *Vásquez & Jaramillo 3443* (MO); provincia Maynas, Negro Urco, Río Napo, 3°00′S, 73°28′W, *Gentry & Emmons 39575* (MO); provincia Maynas, caserío Alianza, Río Tamshiyacu, 4°05′S, 72°58′W, *Gentry et al. 29336* (MO); provincia Maynas, Explorer's Inn, Río Amazonas ca. 2 km W of Indiana, 3°30′S, 73°03′W, *Gentry et al. 54595, 55971, 61789, 65790* (MO), *Grández et al. 3654* (MO), *Vásquez & Jaramillo 16931* (MO); Santa María de Nanay, Quebrada Yarina, *Vásquez & Jaramillo 12224* (MO). **Oxapampa:** Palcazu, Río Alto Iscozacin, Ozuz, 10°19′S, 75°16′W, *Foster & d'Achille 9959* (MO).

Notopleura lanosa C. M. Taylor, sp. nov. TYPE: Colombia. Chocó: municipio de San José del Palmar, Cerro del Torrá, vertiente oriental, trocha arriba del helipuerto, bosque nublado primario, 1940–2160 m, 1 Sep. 1988, *P. A. Silverstone-Sopkin, N. Paz, R. T. González, J. E. Ramos, L. H. Ramos & A. Henao 4968* (holotype, MO-4993720; isotype, CUVC). Figure 4D.

Haec species a congeneris omnibus partibus pubescentia villosa lanosave tectis, stipulis tubularibus atque inflorescentia capitata subcapitatave pedunculata distinguitur.

Terrestrial herbs to 0.5 m tall, unbranched; stems villous to lanose with trichomes 1–2.5 mm long. *Leaf blades* elliptic to ovate, 7–8.5 × 4–5 cm, at apex acute to slightly acuminate, at base obtuse to truncate, drying papyraceous, abaxially paler, on both surfaces moderately to densely lanose with trichomes 1–2.5 mm long, adaxially trichomes sometimes arranged in groups apparently associated with vein areoles, abaxially trichomes sometimes organized in groups following principal veins; secondary veins 9 to 10 pairs, extending nearly to margin, frequently looping to form a submarginal vein closely set to margin; petioles 1.5–3 cm long, villous to lanose; stipules with sheath 3–4 mm long, persistent, truncate, entire, membranaceous, subhyaline except green and opaque along midrib on each interpetiolar section, villosulous to glabrescent throughout but with pubescence denser on midrib, without interpetiolar appendage. *Inflorescences* pseudoaxillary, moderately to densely villous to lanose, deflexed, capitate to subcapitate, with peduncle 5–12 mm long, head turbinate, 10–14 × 15–20 mm; bracts narrowly triangular to narrowly lanceolate, 3–14 mm long, acute, ciliate; *flowers* sessile, with hypanthium villose to villosulous, ca. 0.3 mm long; calyx limb green, externally villosulous, internally glabrous, 3–4 mm long on an individual flower, 5-lobed for ca. ½ its length, lobes triangular, acute, unequal in length on an individual flower, ciliate to densely ciliate; corollas, stamens, and stigmas not seen. *Infructescences* and *fruits* not seen.

Habitat, distribution, and phenology. Northwestern Colombia, in wet forest at 1940–2400 m; collected in young flower bud in August and September.

This species is distinguished by its villous to lanose pubescence on all the vegetative parts, its truncate unappendaged stipule sheaths, its peduncular, capitate to subcapitate inflorescences, and its relatively long calyx limbs. The specific epithet refers to the distinctive pubescence, which is unusual in *Notopleura*.

Paratype. COLOMBIA. **Chocó:** municipio de San José del Palmar, Cerro del Torrá, vertiente oriental, entre el helipuerto y la mesa, *Silverstone-Sopkin et al. 4849* (CUVC, MO).

Notopleura lateralis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria lateralis* Steyerm., Brittonia 33: 391. 1981. TYPE: French Guiana. Sommet des Monts Galbao, à 10 km au SO de Saül, 650 m, 9 Mar. 1975, *J. J. de Granville 2360* (holotype, VEN not seen).

French Guiana.

Notopleura lateriflora (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria lateriflora* Standl., Publ. Field Columbian Mus., Bot. Ser. 7: 100. 1930. TYPE: Colombia. Valle: above La Cumbre, Cordillera Occidental, 1800–2200 m, 14–19 May 1922, *E. P. Killip 5591* (holotype, US-11205731 not seen, microfiche).

Western Colombia to Ecuador.

Notopleura latistipula (Standl.) C. M. Taylor, comb. nov. Basionym: *Cephaelis latistipula* Standl., J. Wash. Acad. Sci. 18: 281. 1928. *Psychotria cartagoensis* Nepokroeff, in Burger & Taylor, Fieldiana, Bot. n.s. 33: 239. 1993, nom. nov. TYPE: Costa Rica. Cartago: vicinity of Orosí, 30 Mar. 1924, *P. C. Standley 39695* (holotype, US-1153168).

Costa Rica.

Notopleura leucantha (K. Krause) C. M. Taylor, comb. nov. Basionym: *Uragoga leucantha* K. Krause, Bot. Jahrb. Syst. 40: 345. 1908. *Cephaelis leucantha* (K. Krause) Standley, Field Mus. Nat. Hist., Bot. Ser. 11(5): 190. 1936. *Psychotria ferreyrae* C. M. Taylor, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1258. 1993. TYPE: Peru. Huánuco: provincia Humalies, inter Monzon et Huallaga, 600–700 m, Sep., *Weberbauer 3668* (holotype, B destroyed photo (F Rockefeller neg. #754) MO).

Colombia to Bolivia.

Notopleura longipedunculata (Dwyer) C. M. Taylor, comb. nov. Basionym: *Psychotria longipedunculata* Dwyer, Ann. Missouri Bot. Gard. 67: 389. 1980, nom. illeg., not *P. longepedunculata* (Gardn.) Muell. Arg., 1881. *Psychotria longipedunculoides* C. M. Taylor, Fieldiana, Bot. n.s. 33: 257. 1993, nom. nov. TYPE: Panama. Veraguas: valley of Río Dos Bocas, 15.6 km NW of Santa Fe, road between Escuela Agrícola Alto Piedra and Calovébora, 450–550 m, 31 Aug. 1974, *T. B. Croat 27621* (holotype, MO-2203532).

Panama to Ecuador.

Notopleura longissima (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria longissima* Standl., Publ. Field Columbian Mus., Bot. Ser. 7: 102. 1930. TYPE: Colombia. Valle: Córdoba, 80–100 m, 6–8 May 1922, *E. P. Killip 5134* (holotype, US-1140557; isotypes, GH not seen, NY not seen).

Panama to western Colombia.

Notopleura macrophylla (Ruiz & Pav.) C. M. Taylor, comb. nov. Basionym: *Psychotria macrophylla* Ruiz & Pav., Fl. peruv. 2: 56, t. 202, fig. a, subfigs. 1–8. 1799. TYPE: Peru. In Peruviae Andium nemoribus versus Pillao ad Iscutunam tractum, *Ruiz & Pavon s.n.* (holotype, presumably MA not seen; isotype, B destroyed photo (Rockefeller neg. # 480) MO).

Psychotria albacostata Rusby, Descr. S. Amer. Pl. 138. 1920. *Psychotria macrophylla* subsp. *albacostata* (Rusby) Steyerm., Mem. New York Bot. Gard. 23: 562. 1972. TYPE: Colombia. Magdalena: Las Nubes, vicinity of Santa Marta, 910–1666 m, 5 Dec. 1898, *H. H. Smith 1806* (holotype, NY not seen; isotype, MO-124226).

Psychotria macrophylla subsp. *anomothyrsa* fo. *tomentella* Steyerm., Mem. New York Bot. Gard. 23: 564. 1972. Syn. nov. TYPE: Ecuador. Esmeraldas: Km 369 on Quito–San Lorenzo railroad, San Lorenzo, 12 July 1964, *C. Játiva & C. Epling 735* (holotype, NY not seen).

Mexico to Bolivia and the Guianas. *Notopleura macrophylla* has been considered one of the most common species of this group and by far the most morphologically variable by previous authors (e.g., Steyermark, 1972). As recently circumscribed, this name has been applied to plants of *Notopleura* with the following combination of characters: well-developed stipule sheaths with conical, succulent, interpetiolar appendages; pseudoaxillary, pedunculate, paniculate inflorescences with the bracts relatively small and not covering the flowers; flowers borne singly or in groups of two to three on dichasial to monochasial axes; calyx limbs 1.5 mm long or shorter; white corollas; and fruits that are usually white with two pyrenes. This group of plants shows notable morphological variation in other features, and a distribution from central Mexico to Bolivia and Venezuela at sea level through high montane forest.

As Steyermark (1972) noted, the original protologue of *Notopleura macrophylla* is problematic in some details. It comprises both a description and a figure, and unfortunately these two elements are inconsistent with each other in details including some that are diagnostic for species of *Notopleura*. One of the inconsistencies is in the form of stipules, which are described as "interfoliaceae, supraxillares, parvae, semiamplexicaules, unidentatae," while they are illustrated as ovate, acute to acuminate, interpetiolar laminas that are half the length of the petioles. The petioles are described as "bipollicans" or ca. 5 cm long, so these stipules are relatively very long for *Psychotria*. The stipule illustrated does not resemble any known species of *Notopleura*, though it is quite similar to the stipules of *P. amplifrons* Standl. (*Psychotria* subg. *Psychotria*), a montane Andean species that has relatively large leaves and paniculate inflorescences that are often displaced to pseudoaxillary in fruit. Another internal conflict in the protologue of *P. macrophylla* is in the length of the inflorescences, which are described as "longitudine petiolorum" (an unusual formulation, which apparently means "equal to" but suggests that possibly a word is missing), while the figure shows the peduncles to be longer than the petioles and the branched portion of the inflorescence to be about four times as long as the petioles. Also, as noted by Steyermark (1972), the fruits of *P. macrophylla* are described as "obscurè violacea," which is problematic because first, this name has always been applied to plants with white or orange fruits, and second, no species of *Notopleura*

are yet known with violet fruits (those of *P. amplifrons* are red). Steyermark (1972: 561–563) did not consider this color description important and concluded that perhaps it was based on the color of dried fruits, although no dried specimens of *Notopleura* have so far been seen with fruits of this color, and none were cited by Steyermark. Considering this fruit color problem together with the other internal conflicts, it seems likely that this protologue was based on specimens or observations of more than one species.

With more collections now available from western South America and in particular Peru, the plants included in *Psychotria macrophylla* by previous authors can be seen to comprise several distinct species. The name is applied here to the plants that match the photo of the isotype specimen, which also constitute most of the plants to which Steyermark (1972) applied this name.

The total range of plants previously included in *P. macrophylla* is here separated among the species listed below. The synonyms listed by Steyermark (1972) are here distributed among these various species, except for *P. nicotianifolia* M. Mart. & Gal. (Bull. Acad. Roy. Bruxelles 11: 229. 1844), which has been treated by more recent authors (e.g., Lorence, 1999) as *Hoffmannia nicotianifolia* (M. Mart. & Gal.) L. O. Wms., and *H. striata* Rusby (Descr. N. Sp. S. Amer. Pl. 134. 1920), which belongs to *Notopleura* but the identity of which is not entirely clear. (Many species of *Hoffmannia* have succulent, divergent, interpetiolar stipules that resemble the interpetiolar stipule appendages of species of *Notopleura*, especially on dried specimens.)

- *N. macrophylla*, with relatively large leaves with the tertiary venation usually noticeably well developed, white fruits, and pyrenes that are triangular to somewhat inflated in cross section with one central ridge, is found from northwestern Colombia to Venezuela and Bolivia;
- *N. steyermarkiana*, with leaves and pyrenes generally similar to those of *N. macrophylla* but with red to orange fruits, is found in the Andes in Venezuela, Colombia, and Ecuador;
- *N. anomothyrsa*, with relatively narrow leaves, corolla lobes that are abaxially appendaged, and mature pyrenes that are strongly dorsiventrally flattened and dorsally 3–5-ridged, is found from southern Mexico to northern Peru, and as circumscribed here does not include the plants treated by Steyermark as *N. macrophylla* subsp. *anomothyrsa* because Steyermark misinterpreted this name;
- *N. tolimensis*, with relatively narrow leaves, corolla lobes that are abaxially smooth, and mature pyrenes that are hemispherical in cross section and dorsally with one or three low ridges, is found from central Mexico to central Peru;
- *N. angustissima*, with relatively narrow leaves, red fruits, and pyrenes that are hemispherical in cross section and dorsally smooth, is found in extreme eastern Panama and presumably adjacent Colombia;
- *N. longissima*, with stipules with expanded interpetiolar laminas and calyx limbs 3–4 mm long, is found from Panama to western Colombia;
- *N. longipedunculata*, with stipules with expanded interpetiolar laminas and calyx limbs 1–1.5 mm long, is found from Panama to Ecuador.

Notopleura macropodantha (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria macropodantha* Standl., Publ. Field Columbian Mus., Bot. Ser. 7: 103. 1930. TYPE: Colombia. Santander: Río Suratá valley, above Suratá, 2000–2300 m, 5–6 Jan. 1927, *E. P. Killip & A. C. Smith 16631* (holotype, US-1352355; isotype, NY not seen).

Colombia.

Notopleura marginata (Benth.) Bullock, Kew Bull. 13: 88. 1958. Basionym: *Coffea marginata* Benth., Pl. hartweg. 193. 1845. *Psychotria hartwegii* Benth., Vidensk Meddel. Nat. Foren. Kjoebenhavn 1852: 37. 1852, not *Psychotria marginata* Sw., 1788. *Psychotria hartwegiana* Standl., Publ. Field Columbian Mus., Bot. Ser. 7: 100. 1930, nom. superfl. TYPE: Colombia. Cauca: ascent to Páramo de Guanacas, *Hartweg 1059* (holotype, K).

Colombia to central Ecuador.

Notopleura micayensis (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria micayensis* Standl., Publ. Field Columbian Mus., Bot. Ser. 7: 105. 1930. TYPE: Colombia. Cauca: La Gallera, Micay Valley, 1200–1500 m, 20–30 June 1922, *E. P. Killip 7760* (holotype, US-1140625).

Costa Rica to Peru. The inflorescence bracts enlarge and darken markedly as the fruits develop, so the infructescences are visually striking and quite different in appearance from the inflorescences. Because of this variation, sometimes flowering specimens have not been recognized as the same species as fruiting specimens.

Notopleura microbracteata (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria microbracteata* Steyerm., Brittonia 33: 391. 1981. TYPE: French Guiana. Sommet des Monts Galbao à 10 km au SO de Saül, 650 m, 9 Mar. 1975, *J. J. de Granville 2362* (holotype, VEN not seen; isotypes, CAY not seen, P not seen).

French Guiana.

Notopleura nepokroeffiae C. M. Taylor, sp. nov. TYPE: Panama. Chiriquí: Palo Alto, 4.5 km NE of Boquete, along western branch of Río Palo Alto, 6300 ft., 24 May 1979, *B. Hammel 7413* (holotype, MO-3014780; isotype, MO-3796484). Figure 5D–F.

Haec species a congeneris foliis nerviis submarginalibus bene evolutis praeditis, limbo calycino 1.2–2 mm longo profunde lobulato, lobulis corollinis appendiculatis atque fructuum maturitate alborum pyrenis dorsiventraliter complanatis crista longitudinali in pagina dorsali munitis distinguitur.

Terrestrial herbs or shrubs to 1 m tall, apparently unbranched; stems glabrous. *Leaf blades* elliptic to usually oblanceolate, 17–30 × 7–14.5 cm, at apex acute to usually acuminate, at base cuneate to acute, drying papyraceous, glabrous or when young sometimes abaxially pustulose, abaxially apparently paler; secondary veins 16 to 22 pairs, uniting in a well-developed submarginal vein; petioles 1–5 cm long, glabrous; stipules with sheath 2–3 mm long, truncate, entire, glabrous, subcoriaceous, with interpetiolar appendage 10–16 mm long, oblanceolate, succulent, somewhat flattened and winged, fimbriate, caducous. *Inflorescences* pseudoaxillary, glabrous to puberulous, paniculate, with peduncle 4–8.5 cm long, branched portion pyramidal, 2–6.5 × 3–7 cm, with developed secondary axes at ca. 3 nodes, these 2 per node; bracts triangular to lanceolate, acute to acuminate, entire, those subtending secondary axes 6–11 mm long, floral bracts 2–4 mm long; *flowers* sessile in glomerules of 5 to 11, with hypanthium glabrous, ca. 0.8 mm long, hemispherical; calyx limb glabrous, 1.2–2 mm long, 5-lobed for ½–¾ its length, lobes triangular; corolla tubular-infundibuliform, white, externally glabrous, internally glabrous except for a pilosulous ring just below mouth of tube, tube ca. 7 mm long, lobes 5, ca. 3 mm long, triangular, abaxially with a conical appendage to 1 mm long; stamens inserted at mouth of corolla tube, anthers ca. 1.2 mm long, exserted; stigmas ca. 0.8 mm long, positioned just below mouth of corolla tube. *Infructescences* similar to inflorescences; *fruits* ellipsoid, ca. 7 × 6 mm, white; pyrenes 2, dorsiventrally flattened, dorsal surface flat with 1 low, sharp, central, longitudinal ridge and margins thickened.

Habitat, distribution, and phenology. Northwestern Costa Rica to western Panama, in wet forest at 1300–2450 m; collected in flower in March–June, in fruit in November.

This species is distinguished by its leaves with well-developed submarginal veins, its calyx limbs 1.2–2 mm long and deeply lobed, its corollas with the lobes abaxially appendaged, its white fruits, and its dorsiventrally flattened pyrenes with one dorsal, central, longitudinal ridge. The specific epithet honors Molly Nepokroeff, who has studied *Notopleura*, particularly in Central America. This new species is similar to *N. submarginalis* and *N. capacifolia*; *N. submarginalis* differs in its larger leaves with rounded to obtuse apices, its larger panicles borne on longer peduncles, its calyx limbs 0.8–1.5 mm long that are only shallowly lobed, and its corolla lobes that are smooth abaxially; *N. capacifolia* differs in its flowers borne singly or in glomerules of two to three along cincinnoid inflorescence axes and its subtruncate to shallowly lobed calyx limbs 0.5–0.8 mm long.

Paratypes. COSTA RICA. **Puntarenas:** Monteverde Biological Reserve, 10°18′30″N, 84°48′W, *Kiehn 880316-1/1* (MO). PANAMA. **Chiriquí:** S slopes of Cerro Pate Macho along Río Palo Alto, 8°47′S, 82°22′W, *Knapp et al. 2093* (MO), *Knapp & Schmalzel 4812* (MO); vicinity of Cerro Punta, above Guadalupe, 8°50′N, 82°35′W, *McPherson 9387* (MO); sendero a la laguna, por el camino a Culebra, Bajo Mono, Boquete, *Montenegro & Cuevas 1154* (MO, PMA).

Notopleura palestinae (Standl. ex Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria palestinae* Standl. ex Steyerm., Acta Biol. Venez. 4: 98, fig. 48. 1964. TYPE: Colombia. Chocó: Río San Juan, cercanías de Palestina, 5–50 m, 12–14 Mar. 1944, *J. Cuatrecasas 16931* (holotype, F not seen; isotype, US-2772235 not seen, microfiche).

Panama to western Colombia. Steyermark's original description of the stipules of this species as simply "bilobed" does not agree with the stipules of the specimens studied, which are united into a sheath with a central, succulent, acute or shortly bifid interpetiolar appendage.

Notopleura panamensis (Dwyer) C. M. Taylor, comb. nov. Basionym: *Montamans panamensis* Dwyer, Ann. Missouri Bot. Gard. 67: 286, fig. 62. 1980. TYPE: Panama. Chiriquí: Cerro Horqueta, 5500 ft., 20 July 1968, *J. D. Dwyer & B. Lallathin 8792* (holotype, MO-2163225).

Psychotria xerococcoides Dwyer, Ann. Missouri Bot. Gard. 67: 441. 1980. Syn. nov. TYPE: Panama. Darién: Cerro Tacarcuna massif between summit camp on westernmost ridge and Pico Tacarcuna, 1650–1800 m, 22 July 1976, *A. Gentry, H. León & L. Forero 16924* (holotype, MO-2465400).

Costa Rica to Panama. These names were published simultaneously, but no differences are evident between their type specimens except the leaf blades are somewhat larger on that of *Montamans panamensis*. The name *M. panamensis* is here chosen because it has been the subject of more study and discussion.

Notopleura parasiggersiana C. M. Taylor, sp. nov. TYPE: Peru. Madre de Dios: provincia de Tambopata, comunidad nativa Infierno, entre Hermosa Chica y Laguna Cococha, 20 Nov. 1988, *C. Díaz & M. Alexiades 3129* (holotype, MO-3707782). Figure 6G–I.

Haec species a congeneris vagina stipulari 0.5–1.5 mm longa, bracteis floralibus 1–2 mm longis, lobulis corollinis breviappendiculatis atque fructuum maturitate rubrorum aurantiacorumve pyrenis in sectione transversali semicircularibus in pagina dorsali laevibus distinguitur.

Terrestrial herbs or subshrubs to 2 m tall, apparently unbranched; stems glabrous. *Leaf blades* elliptic to oblanceolate, 13–30 × 4–10 cm, at apex acute to acuminate, at base acute to attenuate, drying membranaceous, adaxially glabrous, abaxially moderately to densely puberulous becoming glabrescent; secondary veins 7 to 12 pairs, extending nearly to margins; petioles 1–8 cm long, glabrous or usually puberulous becoming glabrescent; stipules with sheath 0.5–1.5 mm long, rounded to broadly triangular, entire, glabrous or puberulous becoming glabrescent, subcoriaceous, with interpetiolar appendage conical, to 2 mm long, caducous. *Inflorescences* pseudoaxillary, puberulous to pilosulous, paniculate, with peduncle 1–5.5 cm long, branched portion pyramidal, 1–4.5 × 1–4.5 cm, with developed secondary axes at 2 to 3 nodes; bracts narrowly triangular to lanceolate, acute, those subtending secondary axes 2–8 mm long, floral bracts 1–2 mm long; *flowers* sessile or subsessile in cymules of 2 to 5, with hypanthium glabrous or puberulous, ca. 1 mm long; calyx limb glabrous or puberulous, 0.8–1 mm long, shallowly 5-lobed, lobes triangular; corolla slenderly infundibuliform, white, externally puberulous to glabrous, internally glabrous, tube ca. 7 mm long, lobes 5, ca. 2 mm long, triangular, abaxially with a conical to linear appendage 0.2–0.6 mm long; stamens inserted in upper part of tube, anthers ca. 1.2 mm long, exserted; stigma ca. 1 mm long, exserted. *Infructescences* similar to inflorescences or sometimes more expanded; *fruits* subglobose, 5–7 mm diam., orange to red; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Amazonian southern Colombia to southeastern Peru, in wet forest at 1–525 m; collected in flower and fruit generally concurrently throughout the year.

This new species is distinguished by its stipule sheaths 0.5–1.5 mm long, its floral bracts 1–2 mm long, its corolla lobes with short abaxial appendages, its orange to red fruits, and its pyrenes that are hemispherical in cross section and smooth on the dorsal surface. It has previously been confused with *Notopleura siggersiana*; *N. siggersiana* differs in its pilosulous or villosulous pubescence on the vegetative structures, its leaves (6)11–17 cm wide, its stipule sheaths 3–5 mm long, and its floral bracts 2–8 mm long. The specific epithet refers to this similarity.

Paratypes. COLOMBIA. **Amazonas:** municipio de Leticia, Parque Nacional Natural Amacayacu, Centro Administrativo Mata-Matá de INDERENA, trocha que conduce de Mata-matá al sector de Amacayacu, 3°47′S, 70°15′W, *Rudas et al. 2879* (COL, MO); Parque Nacional Natural Amacayacu, 3°45′S, 70°15′W, *Vásquez et al. 12658* (MO). PERU. **Loreto:** Prov. Maynas, quebrada de Tahuayo, near Tamashiyaco, *Croat 19578, 19816* (both MO); Río Napo near entrada de Isla Inayuga, *Croat 20545* (MO); 4 km from Mishana, Río Nanay, Estación Biológica Callicebus, 3°55′S, 73°35′W, *Gentry et al. 15805* (MO), *Vásquez et al. 2786* (MO); casería Alianza, Río Tamashiyacu, 4°05′S, 72°58′W, *Gentry et al. 29345* (MO); Mishana, Río Nanay halfway between Iquitos and Santa María de Nanay, 3°50′S, 73°30′W, *Gentry et al. 31732* (MO), *Gentry & Emmons 38781* (MO), *Solomon 3592* (MO); vicinity of Iquitos, *Revilla 3723* (MO); Nauta, carretera Nauta–Iquitos, Km 5, 4°29′S, 73°35′W, *Vásquez & Arevalo 8973* (MO); carretera Iquitos–Nauta, Km 32, *Vásquez & Jaramillo 7850* (MO); Allpahuayo, Km 20 Iquitos–Nauta [road], ca. 35 km SW of Iquitos, 3°50′S, 73°25′W, *Gentry et al. 56019* (MO); 21 km al S de Iquitos, Estación Experimental del Instituto de Investigaciones de la Amazonía Peruana (IIAP), Allpahuayo, 4°10′S, 73°30′W, *Pipoly et al. 12193* (MO), *12261* (MO), *Vásquez & Jaramillo 14073* (MO), *16638* (MO); Quebrada Sucursari, N side tributary of Río Napo, 3°15′S, 72°55′W, *Gentry et al. 42663* (MO), *54457* (MO); región de Amazonas, distrito Las Amazonas, quebrada Sucursari, ExplorNapo Camp, Llachapa, 3°18–20′S, 72°55′W, *Pipoly et al. 13761* (MO), *13935* (MO), *13767* (MO), *14137* (MO), *14503* (MO), *14531* (MO), *Vásquez & Jaramillo 3825* (MO), *Vásquez et al. 16026* (MO), *16096* (MO); Prov. Requena, alrededores de Requena, 5°05′S, 73°50′W, *Vásquez & Jaramillo 4757* (MO); Requena, carretera Requena a Yaquerana, 5°05′S, 73°50′W, *Vásquez 13351* (MO). **Madre de Dios:** Tambopata Nature Reserve, junction of Río La Torre y Río Tambopata, Swamp Trail, 12°50′S, 69°17′W, *Gentry & Jaramillo 57589* (MO); provincia Manu, Manu Park, Cocha Cashu uplands, 11°45′S, 71°00′W, *Núñez 5878* (MO), *6063* (MO); Tambopata Wildlife Reserve, 30 km S of

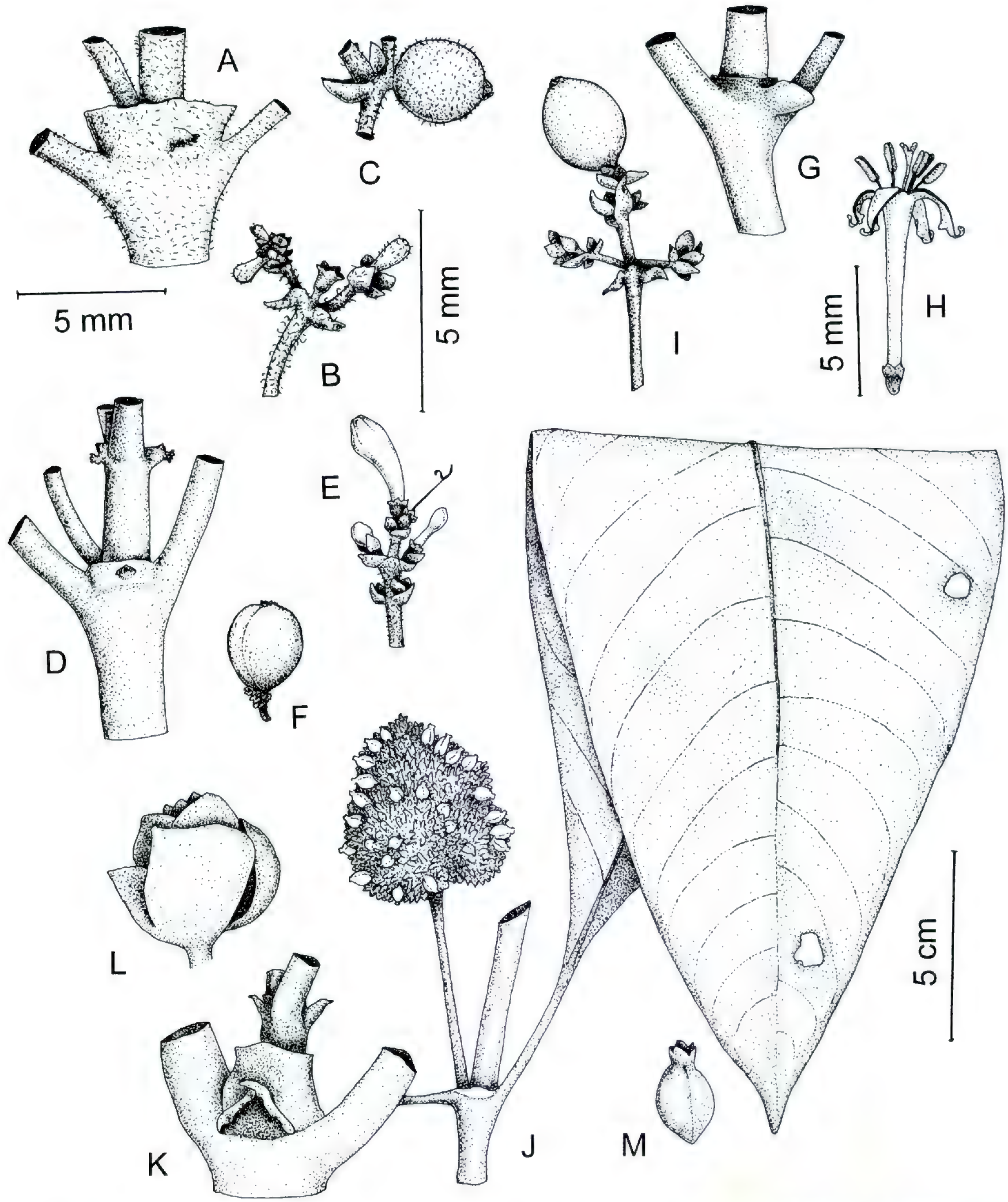


Figure 6. A–C, *Notopleura pilosula* C. M. Taylor. —A. Portion of stem with stipule. —B. Portion of inflorescence with flower buds. —C. Dried fruit. A, B based on *McPherson 12216*; C based on *Hammel et al. 16574*. D–F, *Notopleura scarlatina* C. M. Taylor. —D. Portion of stem with stipules at apex and next proximal node. —E. Portion of inflorescence with flower buds. —F. Dried fruit. D, E based on *Palacios 3062*; F based on *Neill & Cerón 7567*. G–I, *Notopleura parasiggersiana* C. M. Taylor. —G. Portion of stem with stipule. —H. Flower at anthesis. —I. Portion of infructescence with dry fruit. G, H based on *Díaz & Alexiades 3129*; H based on *Knapp 8250*. J–M, *Notopleura pyramidata* C. M. Taylor. —J. Portion of branch with inflorescence. —K. Portion of stem showing stipules at stem apex and next proximal node. —L. Immature inflorescence. —M. Dried fruit. J, M based on *Cogollo et al. 5003*; K, L based on *Fernández A. et al. 9624*. A, D, E, F, G, I, K, L, M to same scale; B, C to same scale.

Puerto Maldonado, 12°15′S, 69°17′W, *Young & Stratton 258* (MO). **Pasco:** provincia Oxapampa, Puerto Laguna, 10°18′S, 75°10′W, *Smith 8405* (MO). **San Martín:** Lamas, Convento, trail to Nuevo Lamas, Km 68 of Tarapoto–Yurimagua road, *Knapp 7950* (MO), *8250* (MO); provincia Mariscal Cáceres, distrito Tocache Nuevo, quebrada de Cascarilla (NE de Puerto Pizana), *Schunke V. 6571* (MO); provincia Mariscal Cáceres, distrito Tocache Nuevo, camino a Santa Rosa (margen derecha del río Mishallo), *Schunke V. 6747* (MO); provincia Mariscal Cáceres, distrito Tocache Nuevo, Cañutillo, NO de la carretera marginal, a 28 km de Tocache, *Schunke V. 7183* (MO); prov. Mariscal Cáceres, distrito Tocache Nuevo, quebrada de Cañuto, cerca a la Chacra de Lizardo Aliaga, *Schunke V. 10935* (MO), cerca a la Chacra del Sr. Victor Arévalo Silva, *Schunke V. 12121* (MO).

Notopleura patria (Standl. & Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria patria* Standley & Steyerm., Fieldiana, Bot. 28: 601. 1953. TYPE: Venezuela. Mérida: Quebrada de Los Saliches, tributary of Río Canaguá, SW of Canaguá, 1950–2135 m, 9 May 1944, *J. A. Steyermark 56411* (holotype, F).

Venezuela.

Steyermark also described one variety of this species, which is provisionally recognized here:

Notopleura patria var. **tovarensis** (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria patria* var. *tovarensis* Steyerm., Mem. New York Bot. Gard. 23: 554. 1972. TYPE: Venezuela. Aragua/Distrito Federal Border: pica hacia Rovar, 1700 m, 1 May 1969, *L. Aristeguieta 7129* (holotype, NY not seen; isotype, VEN not seen).

Venezuela.

Notopleura perparva (Dwyer) C. M. Taylor, comb. nov. Basionym: *Psychotria perparva* Dwyer, Ann. Missouri Bot. Gard. 67: 411. 1980. TYPE: Panama. Darién: Cerro Tacarcuna massif between summit camp on westernmost ridge and Pico Tacarcuna, 1650–1800 m, 22 July 1976, *A. Gentry, H. León & L. Forero 16921* (holotype, MO-2416828; isotype, MO-2416827).

Eastern Panama and presumably in adjacent Colombia. This species is known only from the type, a fruiting specimen collected in a notably poorly known region.

Notopleura pilosula C. M. Taylor, sp. nov. TYPE: Panama/Colombia Border. Darién/Chocó: Parque Nacional del Darién, near gold mine at headwaters of N branch of Río Pocuro, slopes of Cerro Tacarcuna, ca. 6 km N of Cerro Mali, 8°09′N, 77°15′W, 27 Oct. 1987, *B. Hammel, G. de Nevers & H. Herrera 16574* (holotype, MO-3616358). Figure 6A–C.

Haec species a congeneris pubescentia pilosula, stipulis circum caulem in vaginam truncatam connatis, inflorescentia bracteis 1–2 mm longis munita glomerulos 2- vel 3-floros gerente, fructuum maturitate rubrorum pyrenis in sectione transversali semicircularibus in pagina dorsali laevibus distinguitur.

Terrestrial herbs or shrubs to 2 m tall, apparently unbranched; stems pilosulous becoming glabrescent. *Leaf blades* elliptic to rather broadly so, 16–38 × 7–13 cm, at apex acute to usually acuminate, at base acute to cuneate, drying membranaceous to papyraceous, on both surfaces moderately to densely pilosulous; secondary veins 9 to 15 pairs, extending weakly to margins or sometimes looping to interconnect in distal part of blade; petioles 3–7 cm long, pilosulous; stipules with sheath 3–5 mm long, truncate, entire, pilosulous becoming glabrescent, membranaceous, usually deciduous after dis-

talmost 2 to 3 nodes, with interpetiolar appendage conical, 1–2.5 mm long, caducous, apically shortly 2–3-lobed. *Inflorescences* pseudoaxillary, pilosulous, paniculate, with peduncle 1.5–10 cm long, branched portion pyramidal, 3.5–8.5 × 5–8 cm, with developed secondary axes at 2 to 3 nodes, 2 per node; bracts subtending secondary axes triangular to lanceolate, 1–3 mm long, acute, floral bracts suborbicular to ovate, 0.5–1.2 mm long, rounded to obtuse; *flowers* sessile in glomerules of 2 to 3, the glomerules well separated along inflorescence axes; hypanthium glabrous to pilosulous, 0.8–1 mm long; calyx glabrous, limb 0.8–1 mm long, truncate to shallowly 5-lobed, lobes triangular to broadly so; corolla infundibuliform, white, externally densely villosulous or pilosulous becoming glabrescent, internally glabrous, tube ca. 4 mm long, lobes 5, ca. 2.5 mm long, triangular, abaxially smooth; stamens inserted in upper part of tube, anthers ca. 1.2 mm long, exserted; stigmas not seen. *Infructescences* similar to inflorescences or sometimes a little more expanded; *fruits* subglobose to ellipsoid, 4–4.5 × 4–5 mm, red; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Extreme eastern Panama to northwestern Colombia, in wet forest at 600–1600 m; collected in flower in March–

July and October, in fruit in January, April, July, and November.

This species is distinguished by its pilosulous pubescence, its stipules with truncate sheaths and conical, two- to three-lobed, interpetiolar appendages (these are often difficult to discern on herbarium specimens), its inflorescences with floral bracts 0.5–1.2 mm long, its flowers in glomerules of two to three, its red fruits, and its pyrenes that are hemispherical in cross section and smooth on the dorsal surface. The specific epithet refers to the characteristic pubescence found on the vegetative parts of the plant. This new species has been confused with *Notopleura siggersiana*; *N. siggersiana* differs in its lanceolate to ovate bracts 2–6 mm long that completely enclose the flower buds and its inflorescences with a more densely congested arrangement.

Paratypes. PANAMA. **Darién:** Serranía de Pirre, along ascent of Serranía de Pirre above Cana Gold Mine between Río Cana and Río Asucha Ruido, *Croat 37731* (MO), *37823* (MO); middle slopes on W side of Cerro Pirre, *Croat 68927* (MO); Cerro Pirre, ridge top near Rancho Plástico, *Folsom 4267* (MO); Cerro Pirre, valley between Pirre and next most southerly peak, *Folsom 4483* (MO); ridgetop area N of Cerro Pirre, between Cerro Pirre and Rancho Plástico, *Folsom et al. 6338* (MO); Cana-Altos de Nique, trail up ridge between Río Setegantí and Río Alto Tuira (camino a Paleton), SE of goldmine camp and airstrip, *Foster 14130* (MO, PMA); Cuasi–Cana Trail between Cerro Campamento and La Escalera to paramo E of Tres Bocas, *Kirkbride & Duke 1326* (MO); Pirre Massif, Alturas de Nique, above Cana mine, 7°45′N, 77°40′W, *McPherson 12216* (MO). PANAMA/COLOMBIA Border. **Darién/Chocó:** top of Cerro Mali, *Gentry & Mori 13671* (MO); Alto de Nique, southernmost peak of Cerro Pirre Massif, *Gentry et al. 28669* (MO).

Notopleura plagiantha (Standl.) C. M. Taylor, comb. nov. Basionym: *Cephaelis plagiantha* Standl., Field Mus. Nat. Hist., Bot. Ser. 11(5): 190. 1936. *Psychotria plagiantha* (Standl.) C. M. Taylor, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1258. 1993. TYPE: Peru. Loreto: Florida, Río Putumayo, at mouth of Río Zubineta, 200 m, Mar.–Apr. 1931, *G. Klug 2045* (holotype, F-641376; isotype, MO-1005002).

Panama to Bolivia.

Notopleura polyphlebia (Donn. Sm.) C. M. Taylor, comb. nov. Basionym: *Psychotria polyphlebia* Donn. Sm., Bot. Gaz. 33: 253. 1902. TYPE: Costa Rica. Limón: Suerre, Llanuras de Santa Clara, 300 m, Apr. 1896, *J. D. Smith 6854* (lectotype, selected by Lorence (1999), US-943508; isolectotype, US-943509).

Psychotria bullatifolia Standl., Publ. Field Columbian Mus., Bot. Ser. 8(3): 193. 1930. Syn. nov. TYPE: Peru. Loreto: Pebas, on the Amazon River, 27 July 1929, *Ll. Williams 1803* (holotype, F-604504).

Cephaelis bristanii Dwyer & M. V. Hayden, Ann. Missouri Bot. Gard. 55: 34. 1968. Syn. nov. TYPE: Panama. Darién: Cerro Pirre, *N. Bristan 575* (holotype, MO-1961544).

Nicaragua to Peru.

Notopleura pyramidata C. M. Taylor, sp. nov. TYPE: Colombia. Antioquia: municipio de Urrao, Parque Nacional Natural "Las Orquídeas", Sector Calles, margen izquierda de la Quebrada El Guago y margen derecha del Río Calles, 6°32′N, 76°19′W, 1350–1450 m, 6 July 1991, *A. Cogollo, J. G. Ramírez, C. Builes, M. Villa & O. Alvarez 5003* (holotype, MO-4345872; isotype, JAUM). Figure 6J–M.

Haec species a congeneris inflorescentia pedunculata, capitata, pyramidali cylindrica ovoideave atque pyrenis in sectione transversali semicircularibus in pagina dorsali laevibus distinguitur.

Terrestrial herbs or shrubs to 3 m tall, unbranched; stems puberulous to villosulous sometimes becoming glabrescent. *Leaf blades* elliptic to elliptic-oblong, 15–38.5 × 4–16 cm, at apex acute to somewhat acuminate, at base cuneate to acute, drying membranaceous, adaxially moderately or sparsely puberulous to villosulous, often more densely so along margins, abaxially moderately to densely villosulous, paler; secondary veins 12 to 16 pairs, usually extending to unite with margins; petioles 1–3.5 cm long, puberulous to villosulous; stipules with sheath 4–12 mm long, truncate, entire, glabrous to usually puberulous or villosulous, membranaceous, on each interpetiolar portion with a triangular thickened center region and a conical appendage to 1.5 mm long inserted near base of sheath. *Inflorescences* pseudoaxillary, capitate to subcapitate, with peduncle 1.2–6.5 cm long, head pyramidal to cylindrical or ovoid, 15–25 × 15–22 mm; bracts green, villosulous, the outermost lanceolate to ovate, 5–8 mm long, obtuse to acute, ciliolate to entire, floral bracts narrowly triangular to lanceolate, 4–8 mm long, obtuse to acute; *flowers* sessile or subsessile with pedicel or stipe to 1.5 mm long; hypanthium glabrous, ca. 1 mm long; calyx limb glabrous, with tube ca. 2 mm long, lobes 5, narrowly triangular to linear, ca. 1 mm long, separated by concave sinuses, acute, often ciliate; corolla tubular to a somewhat infundibuliform, greenish white, externally glabrous, internally glabrous except for a pilosulous ring just above middle of tube, tube ca. 6 mm long, lobes 5, ca. 2 mm long, triangular, abaxially smooth; stamens inserted in

mouth of tube, anthers ca. 1 mm long, partially exserted; stigmas not seen. *Infructescences* similar to inflorescences or often a little more expanded, to 5 × 4.5 cm; *fruits* ellipsoid, 5–6 × 4–5 mm, orange to red; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Northwestern to central Colombia, in wet forest at 1095–1800 m in the Cordillera Occidental, to 3020 m in the Cordillera Oriental; collected in young flower in July, November, and December, in fruit in January, March–May, and July.

This species is distinguished by its tubular truncate stipule sheaths, its pedunculate, capitate to subcapitate, pyramidal to cylindrical or ovoid inflorescences, and its pyrenes that are hemispherical in cross section and smooth on the dorsal surface. The specific epithet refers to the distinctive inflorescence shape, which is found in several species of *Notopleura* but is particularly striking in *N. pyramidata*.

Paratypes. COLOMBIA. **Antioquia:** municipio de Urrao, zona limotrofe del Parque Nacional Natural "Las Orquídeas", Vereda Calles, margen derecha del río Calles, 6°32′N, 76°19′W, *Cogollo & Ramírez 3126* (JAUM, MO), *17868* (JAUM, MO), margen izquierda del río Calles, *Pipoly et al. 17426* (JAUM, MO); Parque Nacional Natural "Las Orquídeas", Sector Venados arriba, margen izquierda del río Venados, 6°34′N, 76°19′W, *Cogollo et al. 3415* (JAUM, MO); trail from Encarnación to Parque Nacional Natural "Las Orquídeas," western slope of Cordillera Occidental, *Gentry & Rentería 24572* (MO); municipio Frontino, vereda Venados, Parque Nacional Natural "Las Orquídeas", quebrada Las Manzanares, 6°31′N, 76°18′W, *Pipoly et al. 18190* (JAUM, MO), *18237* (JAUM, MO); margen izquierda del río Venado, 6°32′N, 76°19′W, *Pipoly et al. 18165* (JAUM, MO); sitio La Miquera, entre Arenales y río Venados, 6°33′N, 76°19′W, *Pipoly et al. 18298* (JAUM, MO). **Norte de Santander:** municipio de Herrán, Parque Nacional Natural Tama, sector Orocue, hacia el Alto de Pesebre, *Lozano et al. 5521* (COL). **Risaralda:** municipio de Mistrató, corregimiento de Jeguadas, 5°26′N, 76°02′W, *Betancur et al. 3226* (COL, MO); municipio Mistrató, entre los corregimientos de Geguadas y Puerto de Oro, selva de Pisones, *Fernández A. et al. 9624* (COL, HUA).

Notopleura saulensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria saulensis* Steyerm., Brittonia 33: 395. 1981. TYPE: French Guiana. Sommet Monts Galbao à 10 km au SO de Saül, 650 m, 9 Mar. 1975, *J. J. de Granville 2361* (holotype, VEN not seen; isotype, CAY not seen).

French Guiana, perhaps to Bolivia.

Notopleura scarlatina C. M. Taylor, sp. nov. TYPE: Ecuador. Napo: Estación Biológica Jatun Sacha, 01°04′S, 77°37′W, 450 m, 6–14 Oct. 1988, *W. Palacios 3062* (holotype, MO-3678902). Figure 6D–F.

Haec species a congeneris partibus vegetativis glabris puberulisve, vagina stipulari 1–3 mm longa, bracteis floralibus 1–1.2 mm longis, lobulis corollinis appendiculatis atque fructuum maturitate rubrorum aurantiacorumve pyrenis in sectione transversali semicircularibus in pagina dorsali laevibus distinguitur.

Terrestrial herbs or subshrubs to 2 m tall, apparently unbranched; stems glabrous to puberulous. *Leaf blades* elliptic to elliptic-oblong, 20–27 × 8–12.5 cm, at apex shortly acuminate, at base cuneate to attenuate, drying papyraceous, adaxially glabrous, abaxially puberulous to glabrescent; secondary veins 8 to 11 pairs, generally extending to unite with margins; petioles 2–5 cm long, glabrous or puberulous; stipules with sheath 1–3 mm long, truncate to broadly rounded, entire, glabrous to puberulous, subcoriaceous, usually deciduous after distal 1 to 3 nodes, with interpetiolar appendage conical, ca. 1.5 mm long, entire to shortly bifid. *Inflorescences* pseudoaxillary, moderately to densely puberulous, paniculate, with peduncle 2–11 cm long, branched portion pyramidal, 3–11 × 2–12 cm, with developed secondary axes at 3 to 4 nodes, 2 per node; bracts triangular to ovate, acute to obtuse, entire to ciliolate, those subtending secondary axes 2–4 mm long, floral bracts 1–1.2 mm long; *flowers* sessile to subsessile, with hypanthium glabrous to puberulous, ca. 0.8 mm long; calyx limb glabrous to puberulous, 0.2–0.5 mm long, truncate to 5-lobed for ca. ½ its length, lobes deltoid, ciliolate; corolla in bud infundibuliform, white, externally glabrous to puberulous, internally glabrous, tube to 5.5 mm long, lobes 5, to 1.5 mm long, triangular, abaxially smooth; stamens inserted above middle of corolla tube, anthers ca. 1.2 mm long, exserted; stigmas ca. 1 mm long, linear, positioned at mouth of tube. *Infructescences* similar to inflorescences or sometimes more expanded; *fruits* ellipsoid to subglobose, 5–5.5 × 4–5 mm, red to orange; pyrenes 2, hemispherical in cross section, dorsal surface smooth.

Habitat, distribution, and phenology. Known only from the type locality, in wet forest at 400–450 m; collected in flower and fruit generally concurrently, in January, June, and October.

This species is distinguished by its glabrous or puberulous vegetative parts, its stipule sheaths 1–3 mm long, its floral bracts 1–1.2 mm long, its abaxially smooth corolla lobes, its red to orange

fruits, and its pyrenes that are hemispherical in cross section and smooth on the dorsal surface. The specific epithet refers to the red color of the mature fruits. This new species has been confused with *Notopleura siggersiana* of Central America; *N. siggersiana* differs in its pilosulous or villosulous pubescence on vegetative parts, its stipules 3–5 mm long, and its flowers borne in congested glomerules and enclosed by bracts 2–8 mm long.

Paratypes. ECUADOR. **Napo:** Estación Biológica Jatun Sacha, 8 km río abajo de Puerto Misahuallí, 1°04′S, 77°37′W, *Cerón & Iguago 5468* (MO), *Hedin 140* (MO), *Miller et al. 2164* (MO), *Neill & Cerón 7567* (MO).

Notopleura siggersiana (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria siggersiana* Standl., J. Wash. Acad. Sci. 15(13): 289. 1925. TYPE: Costa Rica. Limón: vicinity of Guápiles, 300–500 m, Mar. 1924, *P. C. Standley 37210* (holotype, US-1153058).

Psychotria morii Dwyer, Ann. Missouri Bot. Gard. 67: 400. 1980. TYPE: Panama. Veraguas: 11 km from Escuela Agrícola Alto de Piedra, along Río Dos Bocas, Atlantic slope, 15 Nov. 1974, *S. Mori & J. Kallunki 3139* (holotype, MO-2932202).

Mexico to northwestern Colombia.

Notopleura standleyana (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria standleyana* Steyerm., Fieldiana, Bot. 23: 607. 1953. TYPE: Venezuela. Mérida: Montaña de San Jacinto on N side of Río Chama, opposite Mérida, 1675–2195 m, 19 May 1944, *J. A. Steyermark 56681* (holotype, F).

Venezuela.

Notopleura steyermarkiana C. M. Taylor, sp. nov. TYPE: Venezuela. Yaracuay: Sierra de Aroa, Cerro Negro, 8 km SW of San Felipe, 10°17′N, 69°01′W, 1200–1800 m, 1–2 Apr. 1980, *R. Liesner & A. González 9817* (holotype, MO-2877481). Figure 3D–G.

Haec species a *Notopleura macrophylla* fructuum maturitate rubrorum aurantiacorumve pyrenis laevibus in sectione transversali plerumque triangularibus umbonatisve distinguitur.

Terrestrial herbs or shrubs to 3 m tall, unbranched; stems glabrous. *Leaf blades* elliptic to elliptic-oblong, 20–31 × 9–17 cm, at apex acute to usually acuminate, at base cuneate to rounded, drying papyraceous, glabrous or puberulous; secondary veins 15 to 26 pairs, uniting in a submarginal vein at least in distal part of blade; petioles 2.8–15 cm long, glabrous or puberulous; stipules with sheath 3–5 mm long, truncate to rounded or broadly triangular, entire, glabrous, subcoriaceous, deciduous, with interpetiolar appendage conical, to 3 mm long. *Inflorescences* pseudoaxillary, glabrous to densely puberulous, paniculate, with peduncle 2–6.5 cm long, branched portion pyramidal, 3–17 × 5–15 cm, with developed secondary axes at 2 to 4 nodes, these 2 or 3 per node, dichasial usually becoming cincinnoid with age; bracts triangular, acute, entire, those subtending secondary axes 0.5–2 mm long, floral bracts ovate to suborbicular, acute to rounded, 0.5–1.5 mm long, usually paired and fused at base; *flowers* subsessile, borne singly or in glomerules of 2, with hypanthium puberulous, ca. 0.5 mm long, cylindrical to turbinate; calyx limb puberulous, ca. 0.8 mm long, shallowly 5-lobed, lobes triangular; corolla infundibuliform, white or cream, externally puberulous, internally glabrous except villosulous at stamen attachment, tube ca. 3 mm long, lobes 5, ca. 1.5 mm long, narrowly triangular, abaxially smooth; stamens inserted just above middle of tube, anthers ca. 1.2 mm long, positioned at mouth of tube; stigmas ca. 0.8 mm long, exserted. *Infructescences* similar to inflorescences; *fruits* ellipsoid, 6–9 × 5–7 mm, orange to red; pyrenes 2, when young dorsiventrally flattened with dorsal surface smooth, at maturity somewhat inflated, triangular to umbonate in cross section, dorsal surface flat with 1 usually poorly marked longitudinal ridge, margins not thickened.

Habitat, distribution, and phenology. Venezuela to southern Colombia and Ecuador, in wet forest at 1000–2700 m; collected in flower and fruit throughout the year, usually concurrently.

This species is distinguished by its relatively large leaves with the secondary veins usually united into a clearly evident submarginal vein, its stipules with short sheaths and conical interpetiolar appendages, its inflorescences with the flowers subsessile and generally separated on dichasial branches that become cincinnoid with age, its red to orange mature fruits, and its pyrenes that are smooth and at maturity generally triangular to umbonate in cross section. The specific epithet honors Julian Steyermark, who worked extensively on South American Rubiaceae. *Notopleura steyermarkiana* is very similar to *N. macrophylla*; *N. macrophylla* differs in its corollas with slender tubes 5–7 mm long, its white fruits, and its pyrenes with 4 to 5 low, slender, longitudinal ridges on the dorsal surface. Steyermark (1972: 561–565) treated *N. macrophylla* and *N. steyermarkiana* as subspecies of *Psychotria macrophylla*, because he considered the pyrene shape and fruit color that distinguish

these taxa to be less important than leaf shape and size, which he used to circumscribe *P. macrophylla*. *Notopleura steyermarkiana* generally corresponds to the plants Steyermark called "*Psychotria macrophylla* subsp. *albacostata* (Rusby) Steyerm.," but the type collection of *P. albacostata* belongs to *N. macrophylla* as circumscribed here.

Paratypes. COLOMBIA. **Nariño:** Reserva Natural La Planada, a 7 km de Chuncunés, 1°10′N, 77°58′W, *de Benavides 8969* (MO). **Putumayo:** municipio Mocoa, corregimiento de San Antonio, vereda Alto Campucana, finca La Mariposa, 1°12′N, 76°38′W, *Fernández A. et al. 10759* (COL, MO). ECUADOR. **El Oro:** hacienda Buenaventura, 12 km W of Piñas on road to Machala, 3°48′S, 79°46′W, *Kessler 2589* (MO), *2637* (MO). **Napo:** Archidona Cantón, carretera Hollín–Loreto, Km 25, Sector Challua Yacu, faldas al S del Volcán Sumaco, 1°45′S, 77°38′W, *Cerón & Hurtado 6492* (MO); Cantón El Chaco, Proyecto Hidroeléctrico Coca, Punto ST3, margen derecha del Río Quijos, ca. 10 km al S de Reventador, 00°11′S, 77°39′W, *Palacios 5938* (MO). VENEZUELA. Without locality, *Fendler 545* (MO). **Aragua:** along road from Maracay to Choroní, near Km 23, ca. 1 km N of summit (Altos de Choroní), 9°21′N, 67°24′W, *Croat 54507* (MO); distrito Girardot, Parque Nacional Henri Pittier (Rancho Grande), La Cumbre de Rancho Grande trail, 10°21′N, 67°39′W, *Davidse et al. 16712* (MO). **Lara:** along road between Humocaro Alto and La Palma, 12.5 km above Humocaro Alto, 9°37′N, 70°04′W, *Croat 60635* (MO). **Trujillo:** distrito de Boconó, Guaramacal, 20 km al E de Boconó, 9°14′N, 70°11′W, *Aymard et al. 5242* (MO); distrito de Boconó, Cerro Guaramacal, camino Mosquey–caserío Guaramacal, vertiente noroeste, 9°10′N, 70°11′W, *Stergios et al. 15812* (MO, PORT). **Yaracuay:** distritos Nirgua y San Felipe, Serranía Santa María-Cerro La Chapa, cumbre, al E de la pica Nirgua-Las Marías, 10°15′N, 68°33′W, *Meier 3246* (MO); distrito de Bolívar, entre Las Parchitas, Tierra Fria y Ojo de Agua, 10°20′N, 68°57′W, *Ortega & Smith 2497* (MO); distrito de Urachiche, al N de Urachiche, a lo largo de la carretera entre Buenos Aires y Guamales, parte occidental de la Sierra de Aroa, 10°14′N, 69°01′W, *Steyermark et al. 114093* (MO, VEN).

Notopleura subimbricata (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria subimbricata* Steyerm., Ann. Missouri Bot. Gard. 74: 400. 1987. TYPE: Venezuela. Miranda: Parque Nacional Guatopo, a lo largo del Río Santa Cruz de Río Grande, lado O de la carretera, al SE de Los Alpes, 10°04′45″N, 66°29′30″W, 400 m, 31 Oct. 1981, *J. A. Steyermark & B. Manara 125483* (holotype, VEN not seen).

Venezuela.

Notopleura submarginalis C. M. Taylor, sp. nov. TYPE: Panama. Chiriquí: ridge 8 km N of Los Planes de Hornito, IRHE Fortuna Hydroelectric Project, 8°45′N, 82°12′W, 1250–1300 m, 9 May 1982, *S. Knapp 4988* (holotype, MO-3041787). Figure 5G–J.

Haec species a congeneris foliis grandibus nerviis submarginalibus bene evolutis praeditis, inflorescentia ad apices axium flores in glomerulis gerente pedunculo bene evoluto insidente, limbo calycino 1–1.5 mm longo atque fructuum alborum pyrenis dorsiventraliter complanatis crista longitudinali in pagina dorsali munitis distinguitur.

Terrestrial shrubs or subshrubs to 4 m tall, apparently unbranched; stems glabrous to puberulous usually becoming glabrescent. *Leaf blades* elliptic to broadly elliptic, 23–32 × 16–24 cm, at apex acute to rounded, at base cuneate to truncate, drying papyraceous, on both surfaces glabrous to puberulous, abaxially apparently paler; secondary veins 20 to 23 pairs, uniting in a well-developed submarginal vein; petioles 2.5–7 cm long, puberulous to glabrous; stipules with sheath 3–5 mm long, truncate to broadly triangular, entire, glabrous to puberulous, subcoriaceous, caducous, with interpetiolar appendage to 6 mm long, conical, stout, shortly fimbriate. *Inflorescences* pseudoaxillary, puberulous to pilosulous, paniculate, with peduncle 7–20 mm long, branched portion pyramidal to broadly so, 6–15 × 5.5–24 cm, with developed secondary axes at 3 to 5 nodes, 2 per node; bracts acute, ciliolate, puberulous to villosulous, those subtending secondary axes triangular to lanceolate, 2–10 mm long, floral bracts ovate, 2–3.5 mm long; *flowers* sessile in glomerules of 5 to 11, with hypanthium glabrous to puberulous, turbinate, ca. 1 mm long; calyx limb glabrous or puberulous, 0.8–1.5 mm long, very shallowly 5-lobed, lobes rounded to broadly triangular; corolla infundibuliform, white to cream, externally glabrous or puberulous, internally glabrous except pilosulous at mouth, tube ca. 5 mm long, lobes 5, ca. 2 mm long, triangular, abaxially smooth; stamens inserted just above mouth of corolla tube, anthers ca. 1.2 mm long, exserted; stigmas not seen. *Infructescences* similar to inflorescences; *fruits* ellipsoid, ca. 7 × 6 mm, white; pyrenes 2, dorsiventrally flattened, dorsal surface flat with 1 low, sharp, central, longitudinal ridge and margins thickened.

Habitat, distribution, and phenology. Western Panama, in wet forest at 1000–1300 m; collected in flower in February–July, in fruit in September.

This species is distinguished by its relatively large leaves with well-developed submarginal veins, its inflorescences with well-developed peduncles and the flowers arranged in glomerules at the ends of the axes, its calyx limbs 0.8–1.5 mm long, its white fruits, and its pyrenes that are dorsiventrally flattened with a central longitudinal ridge on the dorsal surface and thickened margins. The specific epithet refers to the distinctive, well-developed submarginal leaf veins. This new species

is similar to *Notopleura capacifolia* and *N. nepokroeffiae*; *N. capacifolia* differs in its inflorescences with the flowers borne singly or in glomerules of two to three along cincinnoid axes and its subtruncate to shallowly lobed calyx limbs only 0.5–0.8 mm long, while *N. nepokroeffiae* differs in its usually smaller leaves that are sharply acuminate at the apex, its inflorescences with smaller panicles borne on shorter peduncles, its calyx limbs 1.2–2 mm long and lobed for ½ or more of their lengths, and its corolla lobes abaxially with well-developed conical appendages.

Paratypes. PANAMA. **Chiriquí:** vicinity of Gulaca ca. 8.5 mi. from Planes de Hornito, La Fortuna on road to dam site, *Antonio 5070* (MO); Fortuna Dam area, along Quebrada Bonito to E of road, 8°45′N, 82°13′W, *Churchill et al. 4732* (MO), *4756* (MO); Fortuna Dam area, Quebrada Bonito to N of reservoir, 8°45′N, 82°13′W, *Churchill 5775* (MO); Fortuna Dam area, between Quebrada Los Chorros and Quebrada Hondo, to N of reservoir, 8°45′N, 82°14′W, *Churchill & Churchill 6178* (MO); along road between Gualaca and Fortuna dam site, 8.3 mi. NW of Los Planes de Hornito, 8°44′N, 82°16′W, *Croat 49967* (MO); along road between Fortuna Lake and Chiriquí Grande, 4.5–5 km N of dam over Fortuna Lake, 8°43′N, 82°17′W, *Croat & Grayum 60042* (MO); ½ km NE of Quebrada de Arena, draining into Río Chiriquí, Carretera del Oleoducto, IRHE Fortuna Hydroelectric Project, 8°46′N, 82°12′W, *Knapp et al. 4052* (MO); forest behind Vivero Forestal, 9 km N of Los Planes de Hornito, IRHE Fortuna Hydroelectric Project, 8°45′N, 82°12′W, *Knapp et al. 4092* (MO); Chiriquí border along Continental Divide on Carretera del Oleoducto ca. 1 km N of Quebrada Arena, IRHE Fortuna Hydroelectric Project, 8°46′N, 82°12′W, *Knapp 5095* (MO); forest behind Vivero Forestal de Boquete, 12 km N of Los Planes de Hornito, IRHE Fortuna Hydroelectric Project, 8°43′N, 82°12′W, *Knapp & Vodicka 5554* (MO); 10 km N of Los Planes de Hornito, IRHE Fortuna Hydroelectric Project, *Knapp & Vodicka 5611* (MO); Continental Divide above Quebrada Arena, Carretera del Oleoducto, IRHE Fortuna Hydroelectric Project, *Knapp & Vodicka 5651* (MO).

Notopleura sucrensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria sucrensis* Steyerm., Mem. New York Bot. Gard. 23: 547. 1972. TYPE: Venezuela. Sucre: laderas que miran hacia La Roma e Irapa, entre la cumbre y La Laguna, NO de Irapa, Cerro de Humo, Peninsula de Paria, 1060–1273 m, 3 Mar. 1966, *J. A. Steyermark 94956* (holotype, VEN not seen).

Venezuela.

Notopleura tapajozensis (Standl.) Bremek., Rec. Trav. Bot. Néerl. 34: 290. 1934. Basionym: *Psychotria tapajozensis* Standl., Publ. Field Columbian Mus., Bot. Ser. 8: 215. 1930. TYPE: Brazil. Pará: San Gabriel, Rio Tapajoz, Jan. 1916, *Kuhlmann 1458* (holotype, B not seen).

Venezuela to northeastern Brazil and Amazonian Colombia. For additional synonymy, see Steyermark (1972).

Notopleura terepaimensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria terepaimensis* Steyerm., Mem. New York Bot. Gard. 23: 565. 1972. TYPE: Venezuela. Lara: Terepaima, 1450 m, 2 Jan. 1954, *B. Trujillo 1955* (holotype, MAR not seen).

Venezuela.

Notopleura thesceloantha (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria thesceloantha* Steyerm., Ann. Missouri Bot. Gard. 75: 347, f. 13. 1988. TYPE: Venezuela. Amazonas: departamento Río Negro, upper Caño Baria, "swampy" area between Río Mawarinuma and headwaters of Río Baria, 00°52′N, 66°15′W, 130 m, 26 Mar. 1984, *R. Liesner 16934* (holotype, MO-3393681).

Colombia to Ecuador and Venezuela.

Notopleura tolimensis (Wernham) C. M. Taylor, comb. nov. Basionym: *Psychotria tolimensis* Wernham, Bull. Misc. Inf. Kew 1914: 68. 1914. TYPE: Colombia. Tolima, Pitalito, Mar. (fl), *Sprague 237* (holotype, K).

Psychotria victoriae Dwyer, Ann. Missouri Bot. Gard. 53: 108. 1966, nom. illeg., not *Ps. victoriae* Standl. (1930). Syn. nov. TYPE: Panama. Chiriquí: Boquete, Collins Finca, Aug. 1964, *Sr. M. V. Hayden 164* (holotype, PMA; isotype, MO-187156A).

Central Mexico to Peru. This species has been included by previous authors in *Psychotria macrophylla*, but with more detailed study of this group of specimens it can be seen to be distinct, as dicussed under *P. macrophylla*. The holotype and isotype collections of *P. victoriae* were both mistakenly deposited at MO; the holotype has more recently been sent to PMA, while the isotype remains at MO.

Notopleura tonduzii (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria tonduzii* Standl., J. Wash. Acad. Sci. 15(13): 287. 1925. TYPE: Costa Rica. Cartago: La Estrella, 26–27 Mar. 1924, *P. C. Standley 39461* (holotype, US-1153160).

Nicaragua to Panama. This species has sometimes been confused with *Notopleura aggregata*.

Notopleura triaxillaris C. M. Taylor, sp. nov. TYPE: Peru. Pasco: Oxapampa Province, Palcazu valley, Río San José in the Río Chuchurras drainage, 10°09′S, 75°20′W, 600 m, 12 May 1983, *D. N. Smith 3942* (holotype, MO-3392740). Figure 3H–J.

Haec species a congeneris vagina stipulari truncata rotundatave 2.5–5 mm longa, inflorescentia sat grandi axes secundarios duos vel plerumque tres ad nodum imum parient atque pyrenis 4–5.5 mm longis dorsiventraliter complanatis crista longitudinali in pagina dorsali munitis distinguitur.

Terrestrial shrubs to 2 m tall, probably unbranched; stems pilosulous or villosulous becoming glabrescent. *Leaf blades* elliptic to elliptic-oblong, 19–32 × 10–15.5 cm, at apex acute to slightly acuminate, at base acute to obtuse or subtruncate, drying membranaceous or papyraceous, on both surfaces glabrous or sparsely to moderately pilosulous or villosulous; secondary veins 12 to 15 pairs, extending to near margins or often looping to form a distinct submarginal vein in distal part of blade; petioles 1.5–5.5 cm long, pilosulous or villosulous becoming glabrescent; stipules with sheath 2.5–5 mm long, truncate to broadly rounded, entire, subcoriaceous, glabrous or villosulous to pilosulous often becoming glabrescent, generally deciduous after distalmost 2 to 3 nodes, with interpetiolar appendage conical, to 3 mm long, entire to bifid, caducous. *Inflorescences* pseudoaxillary, pilosulous or villosulous, paniculate, with peduncle 6–19 cm long, branched portion pyramidal, 5.5–13 × 7–20 cm, with developed secondary axes at 5 to 7 nodes, 2 or usually 3 per node, these subequal or 1 shorter; bracts narrowly triangular, acute, ciliolate, those subtending secondary axes 2–5 mm long, floral bracts 1–2 mm long; *flowers* sessile, separated or in glomerules of 2, usually with arrangement subsecund along inflorescence axes, with hypanthium glabrous or pilosulous, ca. 0.5 mm long; calyx limb glabrous to pilosulous, ca. 1 mm long, subtruncate to shallowly 5-lobed, lobes broadly triangular; corolla tubular-infundibuliform, greenish white or white to cream, externally glabrous, internally glabrous except barbate in throat, tube ca. 4 mm long, lobes 5, ca. 1 mm long, triangular, abaxially smooth or sometimes a little thickened; stamens and stigmas not seen. *Infructescences* similar to inflorescences; *fruits* ellipsoid, 4–5.5 × 4–5 mm, orange; pyrenes 2, dorsiventrally flattened, dorsal surface smooth except with 1 low, sharp, central, longitudinal ridge and margins thickened.

Habitat, distribution, and phenology. Ecuador and Peru, in wet forest at 300–1600 m; collected in flower in February, May, June, and October, in fruit in February, May, and October.

This species is distinguished by its truncate to rounded stipule sheaths 2.5–5 mm long, its relatively large inflorescences with 2 or usually 3 secondary axes produced at the basalmost node, and its dorsiventrally flattened pyrenes 4–5.5 mm long and with 1 central longitudinal ridge on the dorsal surface. The specific epithet refers to its distinctive inflorescence arrangement with 3 secondary axes at the basalmost node; this feature is also found in a few other species of *Notopleura* (e.g., *N. aequatoriana*, *N. cundinamarcana*).

Paratypes. ECUADOR. **Napo:** cantón Archidona, faldas al sur del Volcán Sumaco, carretera Hollín–Loreto, Km 40, Huamaní, 00°43′S, 77°36′W, *Alvarado 23* (MO); carretera Hollín–Loreto, Km 40–50, alrededores de la comunidad Huamaní y del Río Pucuno, 00°43′S, 77°36′W, *Hurtado 869* (MO). PERU. **Amazonas:** provincia de Bagua, distrito de Imaza, Yamayakat, Nororiental del Marañón RENOM, alrededor de la quebrada de Kusu, 4°55′S, 78°19′W, *Quipuscoa 241* (MO). **Huánuco:** hills E of Tingo María, *Croat 21133* (MO); La Divisora, divide between Tingo María and Aguyatia, Cordillera Azul, *Gentry et al. 41436* (MO); provincia de Leoncio Prado, distrito de Rupa Rupa, al E de Tingo María, cerca al Cerro Quemado, *Schunke V. 9947* (MO), *10158* (MO). **Loreto:** provincia de Coronel Portillo, distrito de Padre Abad, la cumbre de la Divisora, *Schunke V. 9856* (MO). **Pasco:** Oxapampa Province, Palcazu valley, Río Cacazu, a few km above confluence with Río Bocaz, near guardpost on road in construction to Puerto Bermúdez, *Foster & Smith 9426* (MO).

Notopleura uberta (Standl. & Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria uberta* Standl. & Steyerm., Fieldiana, Bot. 28: 608. 1953. TYPE: Venezuela. Táchira: near and bordering Quebrada de Palmar, at foot of Páramo de Tamá, 2 km above Bentania and 7 km above Villapaez, 2500 m, 14 July 1944, *J. A. Steyermark 57255* (holotype, F).

Venezuela.

Notopleura uliginosa (Sw.) Bremek., Rec. Trav. Bot. Néerl. 31: 290. 1934. Basionym: *Psychotria uliginosa* Sw., Prodr. 43. 1788. *Uragoga uliginosa* (Sw.) Kuntze, Revis. gen. pl. 2: 963. 1891. TYPE: Jamaica, *Swartz s.n.* (holotype, S not seen).

Mexico and the Antilles to Bolivia and the Guianas. For additional synonymy, see Steyermark (1972: 558).

Notopleura wilburiana (Dwyer) C. M. Taylor, comb. nov. Basionym: *Psychotria wilburiana* Dwyer, Ann. Missouri Bot. Gard. 67: 440. 1980. TYPE: Panama. Veraguas: valley of Río Dos Bocas, 11–13 km beyond Agricultural School at Santa Fe, 350–500 m, 25 July 1974, *T. B. Croat 25745* (holotype, MO-2232437).

Panama to Ecuador. Dwyer originally described the hypanthium as ca. 3 mm long, the calyx teeth as 6, the corolla tube as ca. 3 mm long, and the stipules as "imbricated," but these conditions have not been reconfirmed. The type specimen, which was the only collection cited by Dwyer, has no mature corollas so the corolla tube measurement is questionable, and the only dissection now attached to it is of a young developing fruit. The long hypanthium he described—species of *Notopleura* otherwise have hypanthia 0.5–1.2 mm long—is here interpreted to be a young fruit. All of the flowers seen have only 5 calyx lobes, so the source of that observation is not clear. And, the stipules of this species have interpetiolar laminas that are complanate with respect to each other, though they overlap vertically along the stem so this may be what Dwyer meant by "imbricate."

Notopleura subg. **Viscagoga** (Baill.) C. M. Taylor, comb. nov. Basionym: *Uragoga* sect. *Viscagoga* Baill., Adansonia 12: 227. 1879. *Psychotria* ser. *Viscagoga* (Baill.) Steyerm., Mem. New York Bot. Gard. 23: 566. 1972. TYPE: *Uragoga grosourdieana* Baill. ≡ *Notopleura guadalupensis* (DC.) C. M. Taylor.

Plants succulent to strongly so, epiphytic or sometimes terrestrial growing in saturated soils; stems usually branched, often climbing or clambering. Stipules united around the stem into a truncate, tubular sheath, with interpetiolar appendage conical to reduced or absent. Inflorescences pseudoaxillary or terminal; calyx limb 4- to 5-lobed; corolla 4- to 5(6)-lobed. Fruit red becoming black at maturity; pyrenes 2 to 6, in cross section hemispherical to somewhat dorsiventrally flattened when 2 or triangular when 3 to 6, on the abaxial face smooth or with shallow longitudinal ridges, on the adaxial face smooth or with a central longitudinal ridge.

About 10 species, Mexico and the Antilles to the Guianas, Brazil, and Bolivia, in wet formations from sea level to montane forest.

KEY TO SPECIES OF *NOTOPLEURA* SUBG. *VISCAGOGA*

1a. Leaves 12–40 × 3–30 mm, with the secondary veins hardly or usually not at all evident when dry.
  2a. Leaves elliptic to oblanceolate, obtuse to rounded at apex; inflorescences sessile to subsessile ..... *N. merumensis*
  2b. Leaves elliptic to narrowly lanceolate, acute at apex; inflorescences evidently pedunculate.
    3a. Leaves elliptic to broadly elliptic or lanceolate, 7–30 mm wide, usually not markedly paler abaxially when dry ..... *N. guadalupensis* (p.p.)
    3b. Leaves narrowly elliptic, 3–8 mm wide, often markedly paler abaxially at least when dry ..... *N. maxonii*
1b. Leaves 15 × 10 mm or larger, with the secondary veins usually evident when dry.
  4a. Calyx limb with margin truncate to sinuate ..... *N. multiramosa*
  4b. Calyx limb with margin lobed.
    5a. Fully developed fruit with 4 to 6 pyrenes.
      6a. Inflorescences pyramidal in profile, with axes well developed and spreading at 90° or wider from each other ..... *N. pithecobia*
      6b. Inflorescences corymbiform-rounded in profile, with axes well to poorly developed and ascending at less than 90°.
        7a. Leaves 2.5–5 × 1.5–2.5 cm; Cuba ..... *N. guadalupensis* subsp. *tetrapyrena*
        7b. Leaves 4–8 × 2–3.5 cm; Central and South America.
          8a. Vegetative branches without a leafless stipule sheath at their bases; calyx limbs ca. 1 mm long, puberulous ..... *N. epiphytica*
          8b. Vegetative branches without or often with a leafless stipule sheath at their bases; calyx limbs ca. 0.5 mm long, glabrous except ciliolate on margins ..... *N. peperomiae*
    5b. Fully developed fruit with 2 pyrenes.
      9a. Calyx lobes triangular to narrowly so, acute to acuminate, 0.5–1.5 mm long; corolla 6–7 mm long ..... *N. crassa*
      9b. Calyx lobes broadly triangular and obtuse to deltoid, to 0.8 mm long; corolla 3–5 mm long.
        10a. Calyx limb entire and corolla externally smooth ..... *N. guadalupensis* (p.p.)
        10b. Calyx limb ciliolate or corolla externally papillose-puberulous.
          11a. Calyx limb ciliolate and corollas externally smooth ..... *N. aligera*
          11b. Calyx limb entire and corollas externally papillose-puberulous ..... *N. perpapillifera*

Notopleura aligera (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria aligera* Steyerm., Mem. New York Bot. Gard. 23: 572, fig. 75. 1972. TYPE: Venezuela. Bolívar: cumbre of La Escalera, Río Uiri-yuk, Alto Río Cuyuni, 1000 m, 20–21 Aug. 1962, *B. Maguire, J. A. Steyermark & C. K. Maguire 46872* (holotype, NY not seen microfiche; isotype, VEN not seen).

Southeast Venezuela and Guyana.

Notopleura crassa (Benth.) C. M. Taylor, comb. nov. Basionym: *Psychotria crassa* Benth., J. Bot. (Hooker) 3: 227. 1841. TYPE: "British Guiana" [Venezuela]. Amazonas: Maracoaaca [Marawaca], *Schomburgk s.n.* (holotype, K).

Psychotria crassa fo. *alba* Steyerm., Ann. Missouri Bot. Gard. 71: 331. 1984. TYPE: Venezuela. Bolívar: Piar, Macizo de Chimantá, sector centro-NE del Chimantá-tepui, cabeceras orientales del Caño Chimantá, 5°18′N, 62°09′W, 2000 m, 26–29 Jan. 1983, *J. A. Steyermark, O. Huber & Carreño 128062* (holotype, VEN not seen).

Psychotria crassa fo. *angustior* Steyerm., Ann. Missouri Bot. Gard. 71: 331. 1984. TYPE: Venezuela. Bolívar: Piar, Macizo de Chimantá, sector oriental del Chimantá-tepui, cabeceras del afluente derecho superior del Río Tirica ("Caño del Grillo"), 5°18′N, 62°03′W, 2450 m, 7–9 Feb. 1983, *J. A. Steyermark, O. Huber & Carreño 128992* (holotype, VEN not seen).

Venezuela and the Guianas. Steyermark distinguished *Psychotria crassa* fo. *alba* Steyerm. by its "peduncles, axes of the inflorescence, pedicels, calyx and hypanthium, and fruit completely white," and *P. crassa* fo. *angustior* Steyerm. by its "leaf blades narrowly lanceolate or elliptic-lanceolate and 0.5–1.2 cm wide." However, these characters all vary continuously and without correlation to each other, so these forms are not considered taxonomically meaningful here.

Notopleura epiphytica (K. Krause) C. M. Taylor, comb. nov. Basionym: *Psychotria epiphytica* K. Krause, Verh. Bot. Ver. Prov. Brandenburg 50: 108. 1908. TYPE: Peru. Loreto: in der Cerro de Escalero, 1400 m, Mar. 1903, *E. Ule 6692* (holotype, B not seen, photo (F Rockefeller neg. #462) MO).

Psychotria semimetralis K. Krause, Notizbl. Bot. Gart. Berlin-Dahlem 8: 102. 1922. Syn. nov. TYPE: Peru. Loreto: auf den Bergen östlich von Moyobamba zwischen Hartlaubgehölz, 15–1600 m, 1904, *Weberbauer 4751* (holotype, B not seen).

Psychotria orchidearum Standley, J. Wash. Acad. Sci. 18: 276. 1928. Syn. nov. TYPE: Costa Rica. Heredia: Cerros de Zurquí, NE of San Isidro, 2000–2400 m, 3 Mar. 1926, *P. C. Standley & J. Valerio 50863* (holotype, US-1306503).

Psychotria paramorum Standl., Publ. Field Columbian Mus., Bot. Ser. 7(1): 107. 1930. Syn. nov. TYPE: Colombia. Bolívar: below Páramo de Chaquiño, Cordillera Occidental, 2800–3100 m, 24 Feb. 1918, *E. W. Pennell 4342* (holotype, NY not seen, microfiche).

Psychotria lassula Standl., Publ. Field Columbian Mus., Bot. Ser. 7: 100. 1930. Syn. nov. TYPE: Bolivia. La Paz: North Yungas, Polo-Polo, near Coroico, 1100 m, Oct.–Nov. 1912, *O. Buchtien 5767* (holotype, US-1177869).

Nicaragua to Bolivia and infrequent in the Guianas and Venezuela. This species is more broadly circumscribed here than by previous authors. The plants are variable in particular in leaf size and shape, but no clear patterns are apparent in this variation so several names are here synonymized. No material of *Psychotria dudleyi* Steyerm. (Phytologia 31: 227. 1975) has been seen, but Steyermark considered this similar to *P. epiphytica* and *P. semimetralis*, and it is provisionally referred here also.

Notopleura guadalupensis (DC.) C. M. Taylor, comb. nov. Basionym: *Loranthus guadalupensis* DC., Prodr. 4: 294. 1830. *Psychotria guadalupensis* (DC.) Howard, J. Arnold Arbor. 47: 139. 1966. TYPE: Guadeloupe, *Bertero s.n.* (not seen).

Antilles and Mexico to northern South America; *Notopleura guadalupensis* subsp. *guadalupensis* is found in the Lesser Antilles. For additional synonymy, see Steyermark (1972). Steyermark (1972) recognized four subspecies of *N. guadalupensis*, which he distinguished as follows:

"1. Calyx [limb] subtruncate, the minute projections 0.1 mm long or less
..... *Psychotria guadalupensis* subsp. *tetrapyrena*
1. Calyx [limb] with manifestly developed lobes 0.2–1.5 mm long.
2. Calyx [limb] and hypanthium together 1.5–1.6 mm long; calyx [limb] 0.7–0.8 mm long, the lobes 0.2–0.4 mm long; inflorescence 2–3.5 cm long, 2–7 cm wide
P. guadalupensis subsp. *venezuelica*
2. Calyx [limb] and hypanthium together 2–2.8 mm long; calyx [limb] 1–1.5 mm long, the lobes 0.2–1.5 mm long; inflorescences 1–2 cm long, 1.5–3 cm wide.
3. Corolla 6–7 mm long
P. guadalupensis subsp. *guadalupensis*
3. Corolla 2.5–4 mm long
P. guadalupensis subsp. *grosourdyana*"

These four subspecies are provisionally recognized here, though there appears to be greater variation among and on individual plants in calyx limb length and inflorescence size than Steyermark saw.

Notopleura guadalupensis subsp. **grosourdieana** (Baill.) C. M. Taylor, comb. nov. Basionym: *Uragoga grosourdieana* Baill., Adansonia 12: 227. 1879. *Psychotria pendula* subsp. *grosourdieana* (Baill.) Urb., Symb. Ant. 1: 445. 1900. *Psychotria guadalupensis* subsp. *grosourdieana* (Baill.) Steyerm., Mem. New York Bot. Gard. 23: 570. 1972. TYPE: Puerto Rico. Lares, *Grosourdy s.n.* (holotype, P not seen).

Puerto Rico and the Lesser Antilles. This name has been unnecessarily "corrected" by some authors to "*grosourdyana.*"

Notopleura guadalupensis subsp. **tetrapyrena** (Urb.) C. M. Taylor, comb. nov. Basionym: *Psychotria pendula* subsp. *tetrapyrena* Urb., Symb. Ant. 1: 445. 1900. *Psychotria guadalupensis* subsp. *tetrapyrena* (Urb.) Steyerm., Mem. New York Bot. Gard. 23: 570. 1972. TYPE: Cuba. Pinal de Santa Ana, 800 m, *Wright 249* (holotype, B not seen).

Central America and the Antilles.

Notopleura guadalupensis subsp. **venezuelica** (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria guadalupensis* subsp. *venezuelica* Steyerm., Mem. New York Bot. Gard. 23: 570. 1972. TYPE: Venezuela. Aragua: Rancho Grande, Parque Nacional H. Pittier, Nov. 1962, *G. Agostini & M. Fariñas 30* (holotype, VEN not seen).

South America.

Notopleura maxonii (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria maxonii* Standl., Proc. Biol. Soc. Wash. 37: 53. 1924. TYPE: Costa Rica. Cartago: Santa Clara de Cartago, 20 July 1903, *W. R. Maxon & A. D. Harvey 8250* (holotype, US-1180093).

Costa Rica and Panama.

Notopleura merumensis (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria merumensis* Steyerm., Mem. New York Bot. Gard. 23: 574, fig. 76. 1972. TYPE: Guyana. Upper Mazaruni River Basin, trail along SE ridge, Merumé Mountain, Partang River, 1140 m, 4 July 1960, *S. S. Tillett, C. L. Tillett & R. Boyan 43996* (holotype, NY not seen).

Guyana.

Notopleura multiramosa (Steyerm.) C. M. Taylor, comb. nov. Basionym: *Psychotria multiramosa* Steyerm., Mem. New York Bot. Gard. 23: 568. 1972. TYPE: Guyana. Shoulder of E flank, above Thompson Camp, Mt. Ayanganna, 1418–1525 m, 10 Aug. 1960, *S. S. Tillett, C. L. Tillett & R. Boyan 45079* (holotype, NY not seen).

Guyana and southeastern Venezuela.

Notopleura peperomiae (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria peperomiae* Standl., Contr. U.S. Natl. Herb. 18: 132. 1962. TYPE: Panama. Chiriquí: top of Cerro de la Horqueta, 2265 m, 18 Mar. 1911, *H. Pittier 3235* (holotype, US-677632 not seen, microfiche).

Psychotria orchidearum subsp. *persedens* Dwyer, Ann. Missouri Bot. Gard. 67: 47. 1980. Syn. nov. TYPE: Panama. Panamá: Cerro Campana at 2500 ft., 7 Feb. 1969, *E. L. Tyson 5331* (holotype, MO-1973872).

Costa Rica and western Panama. This is the most commonly collected epiphytic *Notopleura* species in southern Central America.

Notopleura perpapillifera (Steyerm.) C. M. Taylor, comb. nov. *Psychotria pendula* subsp. *trinitensis* Urb., Symb. Ant. 1: 447. 1900. Basionym: *Psychotria perpapillifera* Steyerm., Mem. New York Bot. Gard. 23: 571. 1972, not *Psychotria trinitensis* Urb., 1913. TYPE: Trinidad, *Crueger s.n./Bot. Gard. Herb. 3127* (holotype, B not seen).

Trinidad, Tobago, Venezuela, and Guyana.

Notopleura pithecobia (Standl.) C. M. Taylor, comb. nov. Basionym: *Psychotria pithecobia* Standley, Publ. Field Columbian Mus., Bot. Ser. 8(3): 187. 1930. TYPE: Costa Rica. La Palma, 1550 m, 15 Aug. 1898, *A. Tonduz 12432* (holotype, B not seen; isotype, US).

Costa Rica to Ecuador. The name *Psychotria killipii* Standl. (Publ. Field Columbian Mus., Bot. Ser. 7: 99. 1930) may apply to this species, but the material seen is inadequate for conclusive identification of its identity.

Literature Cited

Andersson, L. & J. H. E. Rova. 1999. The *rps*16 intron and the phylogeny of the Rubioideae (Rubiaceae). Pl. Syst. Evol. 214: 161–186.

Bremekamp, C. E. B. 1934. Notes on the Rubiaceae of Surinam. Rec. Trav. Bot. Néerl. 31: 248–308.

Bullock, A. A. 1958. Nomenclatural notes: V. On the typification of the generic name *Notopleura* (Rubiaceae). Kew Bull. 13: 88.

Lorence, D. 1999. A Nomenclator of Mexican and Central American Rubiaceae. Monogr. Syst. Bot. Missouri Bot. Gard. 73: 1–177.

Nepokroeff, M. 1997. Systematics of the Tropical Shrub Genus *Psychotria* L.: Origins, Speciation, and Breeding Systems in Hawaii. Ph.D. Dissertation, University of Wisconsin-Madison.

———, B. Bremer & K. J. Sytsma. 1999. Reorganization of the genus *Psychotria* and tribe Psychotrieae (Rubiaceae) inferred from ITS and *rbc*L sequence data. Syst. Bot. 24: 5–27.

Steyermark, J. A. 1972. *Psychotria*. *In:* B. M. Maguire & Collaborators, Flora of the Guayana Highlands. Mem. New York Bot. Gard. 23: 406–717.

Taylor, C. M. 1996. Overview of the Psychotrieae (Rubiaceae) in the Neotropics. Opera Bot. Belg. 7: 261–270.

Appendix 1. List of Names and Synonyms of *Notopleura*

Accepted names are presented in Bold. Members of subgenus *Viscagoga* are indicated with an asterisk*. Several synonyms listed below are taken from Steyermark (1972) but not treated in detail in this manuscript.

Cephaelis bristanii Dwyer = **N. polyphlebia**
Cephaelis camponutans Dwyer & M. V. Hayden = **N. camponutans**
Cephaelis gaugeri Dwyer = **N. camponutans**
Cephaelis latistipula Standl. = **N. latistipula**
Cephaelis leucantha (Standl.) K. Krause = **N. leucantha**
Cephaelis plagiantha Standl. = **N. plagiantha**
Coffea marginata Benth. = **N. marginata**
Hoffmannia striata Rusby = *Notopleura* sp. (see discussion under *N. macrophylla*)
Loranthus guadalupensis DC. = **N. guadalupensis***
Montamans panamensis Dwyer = **N. panamensis**
Notopleura acuta C. M. Taylor
Notopleura aequatoriana C. M. Taylor
Notopleura aggregata (Standl.) C. M. Taylor
Notopleura agostinii (Steyerm.) C. M. Taylor
Notopleura albens C. M. Taylor
Notopleura aligera (Steyerm.) C. M. Taylor*
Notopleura aneurophylla (Standl.) C. M. Taylor
Notopleura aneurophylloides (Steyerm.) C. M. Taylor
Notopleura angustissima (Standl.) C. M. Taylor
Notopleura anomothyrsa (K. Schum. & Donn. Sm.) C. M. Taylor
Notopleura araguensis (Steyerm.) C. M. Taylor
Notopleura callejasii C. M. Taylor
Notopleura camponutans (Dwyer & M. V. Hayden) C. M. Taylor
Notopleura capacifolia (Dwyer) C. M. Taylor
Notopleura chapensis (Steyerm.) C. M. Taylor
Notopleura cincinalis C. M. Taylor
Notopleura congesta C. M. Taylor
Notopleura crassa (Benth.) C. M. Taylor*
Notopleura cundinamarcana C. M. Taylor
Notopleura decurrens (Steyerm.) C. M. Taylor
Notopleura discolor (Griseb.) C. M. Taylor
Notopleura dukei (Dwyer) C. M. Taylor
Notopleura epiphytica (K. Krause) C. M. Taylor*
Notopleura fernandezii (Steyerm.) C. M. Taylor
Notopleura guadalupensis (DC.) C. M. Taylor*
Notopleura guadalupensis subsp. **grosourdieana** (Baill.) C. M. Taylor*
Notopleura guadalupensis subsp. **tetrapyrena** (Urb.) C. M. Taylor*
Notopleura guadalupensis subsp. **venezuelica** (Steyerm.) C. M. Taylor*
Notopleura hondurensis C. M. Taylor
Notopleura humensis (Steyerm.) C. M. Taylor
Notopleura iridescens C. M. Taylor
Notopleura lanosa C. M. Taylor
Notopleura lateralis (Steyerm.) C. M. Taylor
Notopleura lateriflora (Standl.) C. M. Taylor
Notopleura latistipula (Dwyer) C. M. Taylor
Notopleura leucantha (K. Krause) C. M. Taylor
Notopleura longipedunculata (Dwyer) C. M. Taylor
Notopleura longissima (Standl.) C. M. Taylor
Notopleura macrophylla (Ruiz & Pav.) C. M. Taylor
Notopleura macropodantha (Standl.) C. M. Taylor
Notopleura marginata (Benth.) Bullock
Notopleura maxonii (Standl.) C. M. Taylor*
Notopleura merumensis (Steyerm.) C. M. Taylor*
Notopleura micayensis (Standl.) C. M. Taylor
Notopleura microbracteata (Steyerm.) C. M. Taylor
Notopleura multiramosa (Steyerm.) C. M. Taylor*
Notopleura nepokroeffiae C. M. Taylor
Notopleura palestinae (Standl. ex Steyerm.) C. M. Taylor
Notopleura panamensis (Dwyer) C. M. Taylor
Notopleura parasiggersiana C. M. Taylor
Notopleura patria (Standl. & Steyerm.) C. M. Taylor
Notopleura patria var. **tovarensis** (Steyerm.) C. M. Taylor
Notopleura peperomiae (Standl.) C. M. Taylor*
Notopleura perpapillifera (Steyerm.) C. M. Taylor*
Notopleura perparva (Dwyer) C. M. Taylor
Notopleura pilosula C. M. Taylor
Notopleura pithecobia (Standl.) C. M. Taylor*
Notopleura plagiantha (Standl.) C. M. Taylor
Notopleura polyphlebia (Donn. Sm.) C. M. Taylor
Notopleura pyramidata C. M. Taylor
Notopleura saulensis (Steyerm.) C. M. Taylor
Notopleura scarlatina C. M. Taylor
Notopleura siggersiana (Standl.) C. M. Taylor
Notopleura standleyana (Steyerm.) C. M. Taylor
Notopleura steyermarkiana C. M. Taylor
Notopleura subimbricata (Steyerm.) C. M. Taylor
Notopleura submarginalis C. M. Taylor
Notopleura sucrensis (Steyerm.) C. M. Taylor
Notopleura tapajozensis (Standl.) Bremek.
Notopleura terepaimensis (Steyerm.) C. M. Taylor
Notopleura thesceloantha (Steyerm.) C. M. Taylor
Notopleura tolimensis (Wernham) C. M. Taylor
Notopleura tonduzii (Standl.) C. M. Taylor
Notopleura triaxillaris C. M. Taylor
Notopleura uberta (Standl. & Steyerm.) C. M. Taylor
Notopleura uliginosa (Sw.) Bremek.
Notopleura wilburiana (Dwyer) C. M. Taylor
Psychotria aggregata Standl. = **N. aggregata**
Psychotria agostinii Steyerm. = **N. agostinii**
Psychotria albacostata Rusby = **N. macrophylla**
Psychotria aligera Steyerm. = **N. aligera***
Psychotria amplinoda Steyerm. = **N. tapajozensis**
Psychotria amplinoda var. *ayangannae* Steyerm. = **N. tapajozensis**
Psychotria aneurophylla (Standl.) Steyerm. = **N. aneurophyl'a**

Psychotria aneurophylloides Steyerm. = **N. aneurophylloides**
Psychotria anomothyrsa K. Schum. & Donn. Sm. = **N. anomothyrsa**
Psychotria araguensis Steyerm. = **N. araguensis**
Psychotria bullatifolia Standl. = **N. polyphlebia**
Psychotria camponutans (Dwyer & M. V. Hayden) Hammel = **N. camponutans**
Psychotria capacifolia Dwyer = **N. capacifolia**
Psychotria caribaea Urb. = **N. discolor**
Psychotria cartagoensis Nepokroeff = **N. latistipula**
Psychotria chapensis Steyerm. = **N. chapensis**
Psychotria crassa Benth. = **N. crassa***
Psychotria crassa fo. *alba* Steyerm. = **N. crassa***
Psychotria crassa fo. *angustior* Steyerm. = **N. crassa***
Psychotria decurrens Steyerm. = **N. decurrens**
Psychotria discolor (Griseb.) Rolfe = **N. discolor**
Psychotria dosbocensis Dwyer = **N. capacifolia**
Psychotria dudleyi Steyerm. = *Notopleura* sp.* (see discussion under *N. epiphytica*)
Psychotria dukei Dwyer = **N. dukei**
Psychotria epiphytica K. Krause = **N. epiphytica***
Psychotria fernandezii Steyerm. = **N. fernandezii**
Psychotria ferreyrae C. M. Taylor = **N. leucantha**
Psychotria guadalupensis (DC.) Howard = **N. guadalupensis***
Psychotria guadalupensis subsp. *grosourdieana* (Baill.) Steyerm. = **N. guadalupensis subsp. grosourdieana***
Psychotria guadalupensis subsp. *tetrapyrena* (Urb.) Steyerm. = **N. guadalupensis subsp. tetrapyrena***
Psychotria guadalupensis subsp. *venezuelica* Steyerm. = **N. guadalupensis subsp. venezuelica***
Psychotria hartwegiana Standl. = **N. marginata**
Psychotria hartwegii Benth. = **N. marginata**
Psychotria humensis Steyerm. = **N. humensis**
Psychotria killipii Standl. = *Notopleura* sp.* (see discussion under *N. pithecobia*)
Psychotria lassula Standl. = **N. epiphytica***
Psychotria laevis DC. = **N. uliginosa**
Psychotria laevis var. *angustifolia* Benth. = **N. uliginosa**
Psychotria lateralis Steyerm. = **N. lateralis**
Psychotria lateriflora Standl. = **N. lateriflora**
Psychotria longipedunculata Dwyer (nom. illeg.) = **N. longipedunculata**
Psychotria longipedunculoides C. M. Taylor = **N. longipedunculata**
Psychotria longissima Standl. = **N. longissima**
Psychotria macrophylla Ruiz & Pav. = **N. macrophylla**
Psychotria macrophylla subsp. *albacostata* (Rusby) Steyerm. = **N. macrophylla**
Psychotria macrophylla var. *angustissima* Standl. = **N. angustissima**
Psychotria macrophylla subsp. *anomothyrsa* (K. Schm. & Donn. Sm.) Steyerm. = **N. anomothyrsa**
Psychotria macrophylla subsp. *anomothyrsa* fo. *tomentella* Steyerm. = **N. macrophylla**
Psychotria macropodantha Standl. = **N. macropodantha**
Psychotria maxonii Standl. = **N. maxonii***
Psychotria merumensis Steyerm. = **N. merumensis***
Psychotria micayensis Standl. = **N. micayensis**
Psychotria microbracteata Steyerm. = **N. microbracteata**
Psychotria morii Dywer = **N. siggersiana**
Psychotria multiramosa Steyerm. = **N. multiramosa***
Psychotria nicotianifolia M. Mart. & Gal. = **Hoffmannia nicotianifolia** (M. Mart. & Gal.) L. O. Wms.
Psychotria orchidearum Standl. = **N. epiphytica***
Psychotria orchidearum subsp. *persedens* Dwyer = **N. peperomiae***
Psychotria palestinae Standl. ex Steyerm. = **N. palestinae**
Psychotria paramorum Standl. = **N. epiphytica***
Psychotria parasitica Sw. (nom. illeg.) = **N. guadalupensis***
Psychotria patria Standl. & Steyerm. = **N. patria**
Psychotria patria var. *tovarensis* Steyerm. = **N. patria var. tovarensis**
Psychotria pendula (Jacq.) Urb. = **N. guadalupensis***
Psychotria pendula subsp. *grosourdieana* (Baill.) Urb. = **N. guadalupensis** subsp. **grosourdieana***
Psychotria pendula subsp. *tetrapyrena* Urb. = **N. guadalupensis** subsp. **tetrapyrena***
Psychotria pendula subsp. *trinitensis* Urb. = **N. perpapillifera***
Psychotria peperomiae Standl. = **N. peperomiae***
Psychotria perpapillifera Steyerm. = **N. perpapillifera***
Psychotria perparva Dwyer = **N. perparva**
Psychotria phytolacca DC. = **N. uliginosa**
Psychotria pithecobia Standl. = **N. pithecobia***
Psychotria plagiantha (Standl.) C. M. Taylor = **N. plagiantha**
Psychotria polyphlebia Donn. Sm. = **N. polyphlebia**
Psychotria saulensis Steyerm. = **N. saulensis**
Psychotria semimetralis K. Krause = **N. epiphytica***
Psychotria siggersiana Standl. = **N. siggersiana**
Psychotria standleyana Steyerm. = **N. standleyana**
Psychotria subimbricata Steyerm. = **N. subimbricata**
Psychotria sucrensis Steyerm. = **N. sucrensis**
Psychotria tapajozensis Standl. = **N. tapajozensis**
Psychotria terepaimensis Steyerm. = **N. terepaimensis**
Psychotria thesceloantha Steyerm. = **N. thesceloantha**
Psychotria tolimensis Wernham = **N. tolimensis**
Psychotria tonduzii Standl. = **N. tonduzii**
Psychotria uberta Standl. & Steyerm. = **N. uberta**
Psychotria uliginosa Sw. = **N. uliginosa**
Psychotria uliginosa var. *discolor* Griseb. = **N. discolor**
Psychotria victoriae Dwyer (nom. illeg.) = **N. tolimensis**
Psychotria wilburiana Dwyer = **N. wilburiana**
Psychotria xerococcoides Dwyer = **N. panamensis**
Uragoga grosourdieana Baill. = **N. guadalupensis** subsp. **grosourdieana***
Uragoga leucantha K. Krause = **N. leucantha**
Uragoga uliginosa (Sw.) Kuntze = **N. uliginosa**
Viscoides pendulum Jacp. = **N. guadalupensis***

Volume 88, Number 3, pp. 373–516 of the ANNALS OF THE MISSOURI BOTANICAL GARDEN
was published on September 17, 2001.

Missouri Botanical Garden Library
3 1753 00301 8519

ANNALS OF THE MISSOURI BOTANICAL GARDEN
AND MISSOURI BOTANICAL GARDEN ANNUAL REPORT
ARE NOW AVAILABLE IN JSTOR!

JSTOR®, a not-for-profit organization, is an important endeavor dedicated to helping the scholarly community take advantage of advances in electronic technologies. The JSTOR Archive makes available the complete backfiles of *Annals of The Missouri Botanical Garden*, *Missouri Botanical Garden Annual Report (1890-1912)*, and more than 100 other scholarly journals to researchers through participating libraries.

JSTOR has recently announced the release of its Ecology & Botany Collection. Developed in cooperation with the Ecological Society of America, the Collection includes the archives of twenty-nine journals, offering online access to more than 1 million pages of important historic literature.

The Ecology & Botany Collection

American Journal of Botany
American Midland Naturalist
American Naturalist
Annals of the Missouri Botanical Garden
Annual Review of Ecology and Systematics
Biotropica
Brittonia
Conservation Biology
Diversity and Distributions
Ecological Applications
Ecological Monographs
Ecology
Evolution
Functional Ecology
Global Ecology and Biogeography Letters
International Journal of Plant Sciences
Journal of Animal Ecology
Journal of Applied Ecology
Journal of Biogeography
Journal of Ecology
Journal of Tropical Ecology
Journal of The Torrey Botanical Society
Limnology and Oceanography
Missouri Botanical Garden Annual Report
New Phytologist
Paleobiology
Quarterly Review of Biology
Systematic Biology
Systematic Botany

Information regarding JSTOR is available at http://www.jstor.org
120 Fifth Avenue, New York, NY 10011

CONTENTS

Phylogeny and Subfamilial Classification of the Grasses (Poaceae) ——— *Grass Phylogeny Working Group* 373

Phylogeny, Radiation, and Transoceanic Dispersal of New Zealand Alpine Buttercups: Molecular Evidence under Split Decomposition ——— *Peter J. Lockhart, Patricia A. McLenachan, David Havell, David Glenny, Daniel Huson & Uwe Jensen* 458

Overview of the Neotropical Genus *Notopleura* (Rubiaceae: Psychotrieae), with the Description of Some New Species ——— *Charlotte M. Taylor* 478

Cover illustration. Top: *Pronuba yuccasella*, female, in the act of gathering pollen from the anthers of *Yucca filamentosa*. Bottom: Pistils of *Y. filamentosa*, showing, from left, a deformed specimen and varying lengths of the stamens. From *Annual Rep. Missouri Bot. Gard.* 1892, The Yucca Moth and Yucca Pollination, by C. V. Riley. See Coevolution, the 40th Annual Systematics Symposium of the Missouri Botanical Garden: Introduction, first issue of this volume.

QK
.A47
c.1

Annals of the Missouri Botanical Garden

2001

Volume 88
Number 4

Volume 88, Number 4
Fall 2001

Annals of the
Missouri Botanical Garden

The Annals, published quarterly, contains papers, primarily in systematic botany, contributed from the Missouri Botanical Garden, St. Louis. Papers originating outside the Garden will also be accepted. All manuscripts are peer-reviewed by qualified, independent reviewers. Authors should write the Managing Editor for information concerning arrangements for publishing in the ANNALS. Instructions to Authors are printed in the back of the last issue of each volume and are also available online at www.mobot.org/mbgpress.

Editorial Committee

Victoria C. Hollowell
Editor,
Missouri Botanical Garden

Amy Scheuler McPherson
Managing Editor,
Missouri Botanical Garden

Diana Gunter
Associate Editor,
Missouri Botanical Garden

Aida Kadunic
Senior Secretary

Barbara Mack
Administrative Assistant

Ihsan A. Al-Shehbaz
Missouri Botanical Garden

Gerrit Davidse
Missouri Botanical Garden

Roy E. Gereau
Missouri Botanical Garden

Peter Goldblatt
Missouri Botanical Garden

Gordon McPherson
Missouri Botanical Garden

P. Mick Richardson
Missouri Botanical Garden

Henk van der Werff
Missouri Botanical Garden

For subscription information contact ANNALS OF THE MISSOURI GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897. Subscription price for 2002 is $140 per volume U.S., $150 Canada & Mexico, $175 all other countries. Four issues per volume. The journal *Novon* is included in the subscription price of the ANNALS.

annals@mobot.org (editorial queries)
http://www.mobot.org/mbgpress

THE ANNALS OF THE MISSOURI BOTANICAL GARDEN (ISSN 0026-6493) is published quarterly by the Missouri Botanical Garden, 2345 Tower Grove Avenue, St. Louis, MO 63110. Periodicals postage paid at St. Louis, MO and additional mailing offices. POSTMASTER: Send address changes to ANNALS OF THE MISSOURI BOTANICAL GARDEN, % Allen Marketing & Management, P.O. Box 1897, Lawrence, KS 66044-8897.

© Missouri Botanical Garden 2001

The mission of the Missouri Botanical Garden is to discover and share knowledge about plants and their environment, in order to preserve and enrich life.

This paper meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

Volume 88
Number 4
2001

Annals
of the
Missouri
Botanical
Garden

THE RENAISSANCE OF THE APOCYNACEAE S.L.: RECENT ADVANCES IN SYSTEMATICS, PHYLOGENY, AND EVOLUTION: INTRODUCTION[1]

Mary E. Endress[2] *and*
W. Douglas Stevens[3]

Abstract

Since Robert Brown split the Asclepiadaceae out of Jussieu's Apocynaceae nearly 200 years ago, these two groups have traditionally been maintained as two separate but closely related families, despite some dissention among specialists. Over the past 15 years, a wealth of new data, especially that generated by molecular and cladistic studies, supports earlier results based on morphology, that the traditional classification is artificial in various aspects and at several hierarchical levels. In many cases convergence and classifications based mainly on a single character have long led us astray, and in part, we have been reluctant to relinquish long-cherished ideas. However, the biggest changes are due to new concepts such as clades, monophyly, and paraphyly, which were not around in Brown's day, and which have fundamentally changed the way we perceive taxonomic relationships today. The collection of papers presented here are based on the papers and posters presented at the symposium "Evolution and Phylogenetics of the Apocynaceae s.l.," a part of the 16th International Botanical Congress held in 1999 in St. Louis, Missouri. They provide a representative cross section of the most recent findings, touching on all higher taxonomic levels, and based on a variety of different techniques, of this complex and fascinating group.

Key words: Apocynaceae, Asclepiadaceae, clade, classification, monophyly, paraphyly, phylogeny, systematics.

In contrast to Jussieu's (1789) original all-inclusive delimitation of the Apocynaceae, until just a few years ago the Asclepiadaceae (and sometimes also the Periplocaceae) were treated as separate families. With the application of cladistic and molecular approaches to plant systematics, the study of the relationship between the Apocynaceae and Asclepiadaceae, after having stagnated for at least 50 years, has become a hot topic for phylogenetic and evolutionary investigations. Just in the past five years great strides have been made in elucidating the phylogeny of the group.

[1] We thank the contributors for their involvement in the symposium and all their hard work and cooperation in the assembling of the symposium proceedings. Victoria Hollowell provided guidance and support at every phase, for which we are grateful. Thanks also go to the reviewers, whose critiques improved the papers.

[2] Institute of Systematic Botany, The University of Zürich, Zollikerstrasse 370, 8008 Zürich, Switzerland.

[3] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, U.S.A.

The forerunners of this resurgence of interest in the relationships between the Apocynaceae and the Asclepiadaceae were two papers, neither of which was focused on the Apocynaceae s.l., but both of which contained a cladistic analysis of Apocynaceae and Asclepiadaceae based on morphological characters. Both papers included only nine ingroup taxa, together with Loganiaceae/Gentianaceae or Loganiaceae alone as the outgroup. The earlier of the two, by Wanntorp (1988), pointed out that Apocynaceae s. str. are paraphyletic, and thus proposed inclusion of Asclepiadaceae within Apocynaceae. In addition, it questioned Schumann's (1895) interpretation of pollinium orientation in Asclepiadaceae, underscoring doubts raised earlier by Demeter (1922) and Safwat (1962). Schumann (1895) considered the taxa with pendent pollinia (the Asclepiadeae) to be the most primitive in the Asclepiadoideae, and those with erect pollinia as the "crowning glory" of the entire family. In contrast, in Wanntorp's (1988) analysis, the Asclepiadeae came out as the crown clade, suggesting that erect orientation of pollinia is ancestral, rather than derived, and that pendent orientation is an apomorphy of the Asclepiadeae, making them the most derived tribe of the asclepiads. This position is supported in the contribution by Liede (2001) in this volume.

The second paper, by Judd et al. (1994), also recognized that the Apocynaceae s. str. are paraphyletic and proposed amalgamation of Apocynaceae and Asclepiadaceae in order to make the group monophyletic. In addition, this was the first paper to suggest that the congenitally syncarpous ovary in *Carissa* may not be the ancestral condition in the family, as had been proposed in all traditional classifications (e.g., Schumann, 1895; Leeuwenberg, 1994). This hypothesis was confirmed for *Acokanthera* two years later based on molecular (Sennblad & Bremer, 1996) as well as combined morphological and molecular (Endress et al., 1996) data.

The first important paper contributing toward the rebirth of the Apocynaceae s.l., and dealing explicitly with the family, was that by Sennblad and Bremer (1996), which was based on sequence data of the *rbc*L gene for 24 taxa of Apocynaceae s.l. The results of this study indicated that the Asclepiadaceae are nested within the Apocynaceae. Recognition of Asclepiadaceae as a separate family would result in a paraphyletic Apocynaceae. Further, even monophyly of the traditional Asclepiadaceae was questioned, since *Parquetina* (= *Periploca*) (Periplocoideae) came out as sister to *Prestonia* (Apocynoideae). The authors recommended the amalgamation of the two families. In addition, weaknesses in the traditional classification of Apocynaceae s. str. were indicated. One of the most notable results was the position of *Alstonia*, as the basalmost taxon in the Apocynaceae, which was the first well-supported crack in the veneer of the traditional classifications of Apocynaceae s. str., all of which considered the syncarpous Carisseae to be at the base of the family. *Acokanthera* and *Pictralima* (both Carisseae in traditional classifications), on the other hand, came out significantly higher on the tree.

Swarupanandan et al., also in 1996, published a classification, which incorporated new morphological characters, such as whether there was a distinct style between the ovaries and the stylehead, and the location of attachment of pollinia to caudicles (rather than the orientation of the pollinia, as in earlier classifications). They proposed a controversial new subdivision of the Asclepiadaceae, which recognized three tribes: Secamoneae, Stapelieae (= Ceropegieae), and Asclepiadeae. Marsdenieae were included in Stapelieae, and Gonolobeae were included in Asclepiadeae. Periplocoideae were excluded from Asclepiadaceae and treated as a separate family. Most subsequent analyses support the inclusion of Gonolobeae in Asclepiadeae and do not refute including Marsdenieae in Ceropegieae, whereas the relationships of Periplocoideae remain unresolved.

The next paper to suggest that all was not rosy in the traditional classifications of Apocynaceae s. str. was that by Endress et al. (1996), which was a combined analysis including *rbc*L and *mat*K sequences, as well as 48 morphological characters for 14 genera of Apocynaceae s. str. and several outgroup genera from various families of the Gentianales. The results of this study agreed overall with those of Sennblad and Bremer (1996).

In 1998 Sennblad et al. published a paper focused on the Wrightieae (sensu Leeuwenberg, 1994), in which sequence data of the *rbc*L gene and 25 morphological characters for 21 taxa of Apocynaceae s.l. were cladistically analyzed. The results suggested that in the classification of Apocynaceae s. str. by Leeuwenberg (1994), not only are the Wrightieae and its subtribes paraphyletic, but so are all of the other tribes of higher Apocynoideae. As in their previous paper (Sennblad & Bremer, 1996), *Periploca* grouped with taxa of the Apocynoideae, rather than with *Secamone* (Asclepiadaceae). Perhaps most surprising of all was the position of *Baissea* (Apocynoideae) as a sister group to *Secamone*.

Focusing on the basal clades of the former Asclepiadaceae, Civeyrel et al. (1998) studied 46 taxa using the gene *mat*K, and then compared the phylogeny obtained with palynological characters that have traditionally been used to delimit subfamilies and tribes. In this phylogeny Asclepiadaceae (including Periplocoideae, Secamonoideae, and Asclepiadoideae) were monophyletic. However, none of the crucial taxa of the higher Apocynoideae were included, thus reducing the significance of this analysis as to predicting whether or not the traditional Asclepiadaceae are monophyletic. Within the Asclepiadoideae, *Fockea* was found to be sister to the rest of the Asclepiadoideae, supporting recognition of Fockeeae as a separate tribe of Asclepiadoideae, as had been proposed previously by Kunze et al. (1994) based on morphological evidence. The analysis also showed that parallelisms have occurred in a number of the palynological characters traditionally used to delimit tribes.

In 2000 Sennblad and Bremer expanded their original *rbc*L-based study from 1996 to include 77 taxa of Apocynaceae s.l. The results were very similar to their previous *rbc*L analysis. The six included taxa of Periplocoideae formed a clade with the three taxa of the Echiteae (higher Apocynoideae), whereas *Baissea* (higher Apocynoideae) was intercalated between Secamonoideae and Asclepiadoideae. *Cibirhiza* (the other genus of Fockeeae) came out as sister to the rest of the Asclepiadoideae, providing additional support for recognition of the tribe Fockeeae.

Most recently, Liede and coworkers (e.g., Liede, 2001; Liede & Täuber, 2000; Liede et al., in press) have focused on the subtribal classification within the large tribe Asclepiadeae, which, judging by the results of these papers, is also sorely in need of revision.

It seems clear now that the Apocynaceae and Asclepiadaceae are best united into a single family. Repeatedly, morphological characters assumed to be stable have turned out to be parallelisms, with the result that long-standing taxa are no longer valid, or traditionally recognized taxa have been found to be grades rather than clades (Goyder, 1999). A new classification of Apocynaceae s.l. was recently published by Endress and Bruyns (2000), which recognized five subfamilies: Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. The delimitation and relationships among the tribes therein are still unresolved, as is the position of Periplocoideae (i.e., the question of monophyly of Asclepiadaceae).

The 16th International Botanical Congress held in August of 1999 in St. Louis, Missouri, was thus an opportune time for a symposium on Apocynaceae s.l., in which systematists, employing various disciplines and working on different subfamilies or tribes or with taxa from different geographic regions, could come together and present their results and exchange ideas. The goals were to achieve a better understanding of the evolution of these highly specialized flowers and to develop a more collaborative effort toward a consensus classification of the group.

Progress has been made in our efforts to come up with a consensus classification. Most specialists in the family now accept the five subfamilies given in Endress and Bruyns (2000): Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae. We are aware that the first two subfamilies are paraphyletic, but until more data are available, recognition of these two subfamilies seems to be the most prudent avenue to follow. Based on the analyses presented in this volume, the Secamonoideae could also be interpreted as a tribe of the Asclepiadoideae. However, the Secamonoideae are characterized by a number of distinctive morphological characters, and it might be rash to reduce them to a tribe until more information is available. Tribal delimitation is more problematic, especially in the two basalmost subfamilies, Rauvolfioideae and Apocynoideae. The tribes presented in Endress and Bruyns (2000) for these two basalmost subfamilies (nine tribes in Rauvolfioideae and five tribes in Apocynoideae) are currently the best that we have. Both Periplocoideae and Secamonoideae are probably best left without delimitation into tribes at present. Asclepiadoideae, which are by far the largest subfamily, are considered to comprise four tribes: Fockeeae, Marsdenieae, Ceropegieae, and Asclepiadeae (including Gonolobeae). Although the two genera of the Fockeeae (*Cibirhiza* and *Fockea*) were included in Marsdenieae by Endress and Bruyns (2000), recognition of Fockeeae as their own tribe is supported in all analyses of asclepiads presented in this volume. Other interpretations of tree topologies within the Asclepiadoideae could be the inclusion of the Marsdenieae in the Ceropegieae (as proposed by Swarupanandan et al., 1996). Conversely, the Gonolobeae could be reinstated as a separate tribe. Below the tribal level the situation is far too nebulous to attempt to provide a unified classification, though Liede continues to work on the relationships in the large tribe Asclepiadeae (Liede, 2001; Liede & Täuber, 2000; Liede et al., in press).

The seven papers that follow are the result of the symposium, entitled "Evolution and Phylogenetics

of the Apocynaceae s.l." In these papers, subfamilies and tribes follow this classification outline:

RAUVOLFIOIDEAE Kostel.
- Alstonieae G. Don
- Vinceae Duby
- Willughbeieae A. DC.
- Tabernaemontaneae G. Don
- Melodineae G. Don
- Hunterieae Miers
- Plumerieae E. Mey.
- Carisseae Dumort.
- Alyxieae G. Don

APOCYNOIDEAE Burnett
- Wrightieae G. Don
- Malouetieae Müll.-Arg.
- Apocyneae Rchb.
- Mesechiteae Miers
- Echiteae Bartl.

PERIPLOCOIDEAE R. Br. ex Endl.

SECAMONOIDEAE Endl.

ASCLEPIADOIDEAE R. Br. ex Burnett
- Fockeeae Kunze, Meve & Liede
- Marsdenieae Benth.
- Ceropegieae Orb.
- Asclepiadeae (R. Br.) Duby

The first paper, by Potgieter and Albert (2001), probes the phylogenetic relationships of the Apocynaceae s.l., using plastid genome sequences of the *trn*L intron and *trn*L-F spacer. This represents the most comprehensive phylogenetic study of the Apocynaceae s.l. to date, including sequences for 152 accessions (75 Apocynaceae and 48 Asclepiadaceae), and representatives of all subfamilies and tribes recognized in Endress and Bruyns (2000). This work was undertaken mainly to address the lack of adequate sampling in earlier studies, especially with regard to the basalmost clades in the family. The results in most cases are similar to those in the previous studies by Sennblad and Bremer (1996, 2000), Sennblad et al. (1998), and Civeyrel et al. (1998), but provide better resolution, especially within the Rauvolfioideae, and for the placement of several genera, whose systematic position has been in dispute (e.g., *Haplophyton, Vallesia, Microplumeria, Laxoplumeria*). Surprises include the number of additional taxa at the very base of the tree, below *Alstonia,* which was the basalmost genus in the family in earlier analyses (Sennblad & Bremer, 1996, 2000; Endress et al., 1996; Civeyrel et al., 1998), demonstration of polyphyly of the Carisseae, Plumerieae, and Alyxieae sensu Leeuwenberg (1994), evidence for the multiple evolution of seed wings in various tribes of the Rauvolfioideae, and support for the recognition of *Tonduzia.* The classification published by Endress and Bruyns (2000), and the large phylogenetic analysis of Apocynaceae s.l. by Potgieter and Albert (2001) published in this volume, did not take place in isolation but evolved through reciprocal exchange of information. In large part the classification of Endress and Bruyns (2000) was made more natural due to the inclusion of (at that time unpublished) results of the study by Potgieter and Albert (2001), generously provided by the authors.

The second paper, by Venter and Verhoeven (2001), focuses on the Periplocoideae. Although small in number of species (only 31 genera and 181 species), the poorly known Periplocoideae are at present the most enigmatic group of the Apocynaceae s.l. in terms of their systematic position. In terms of complexity they fall somewhere between the Apocynoideae and the Secamonoideae. Periplocoideae have always been the odd man out because their translator structure appears so different from that of the higher asclepiads, although there are actually more similarities in the translator structure of the two groups than may be apparent at first sight (Schick, 1982; Endress, in press). Their systematic position in the Apocynaceae s.l. remains uncertain: either they are the basal clade of the traditional Asclepiadaceae, or they form a clade separate from the Secamonoideae + Asclepiadoideae, but also nested in the higher Apocynoideae. In their paper Venter and Verhoeven (2001) provide an overview of the subfamily, including new synonymies and a key to the genera; a cladistic analysis based on morphological characters is also presented.

In the third paper, Verhoeven and Venter (2001) compare pollen morphology of Periplocoideae, Secamonoideae, and Asclepiadoideae. Traditionally, Periplocoideae were distinguished from Secamonoideae and Asclepiadoideae by having pollen in tetrads, whereas the other two subfamilies have pollinia. Recently, however, the authors have shown that some genera of Periplocoideae also have their pollen coalesced into pollinia (Verhoeven & Venter, 1998). In their current paper more taxa are added to the list. Each anther in Periplocoideae still has four pollen sacs (the ancestral state in the family) and thus in genera with pollinia, four pollinia are produced in each anther, as in Secamonoideae. This distinguishes Periplocoideae and Secamonoideae from the Asclepiadoideae, in which, during evolution, the two dorsal pollen sacs in the anther have been lost, so that each anther only produces two pollinia. The pollinia in Periplocoideae are also similar to those in Secamonoideae in that they are not covered by an outer pollinium wall, but are

composed merely of agglutinated tetrads. There are, however, differences between the two groups in the internal structure of the pollinia. Pollinia in Asclepiadoideae, in contrast, are composed of single grains, covered by an outer pollinium wall. The only exception known is *Fockea*, which has pollinia similar to those found in Secamonoideae and Periplocoideae. The unresolved position of Periplocoideae in Apocynaceae s.l. raises the question of whether pollinia have evolved independently in Periplocoideae and again in the Secamonoideae + Asclepiadoideae or whether they are homologous in these two groups. Results presented in this paper suggest that pollinia may have arisen twice within the Periplocoideae alone.

The fourth paper, by Civeyrel and Rowe (2001), explores phylogenetic relationships between and within the genera of Secamonoideae, as well as with other subfamilies, based on the plastid gene *mat*K. With only seven recognized genera and under 200 species, more than half of which belong to the genus *Secamone*, Secamonoideae are the smallest subfamily of Apocynaceae s.l. Like the Periplocoideae, the Secamonoideae are restricted to the Old World and are also poorly collected. Madagascar is the main center of speciation and contains half the known genera and species. Secamonoideae exhibit characteristics that are a mosaic of those from Asclepiadoideae on the one hand, and the more basal subfamilies of Apocynaceae s.l. on the other, and are thus one of the crucial groups in understanding the relationships within the family.

In the fifth paper, Fishbein (2001) plots various scenarios of diversification of two key characters in the former Asclepiadaceae, the pollinarium and the corona, onto a phylogenetic framework based on sequences of the plastid gene *mat*K. One of the most difficult tasks in the Apocynaceae s.l. is distinguishing homology from convergence. A major focus of continued dispute is the assessment of homology of various types of coronas. The analyses presented here suggest that evolution of pollinaria has been relatively conserved, whereas evolution of coronas, especially gynostegial coronas, which characterize the Asclepiadoideae, is marked by radiation and diversification. Since a gynostegial corona is taken to be the ancestral state in Asclepiadoideae, its absence in any taxa in this subfamily must be considered to be derived. In this study, independent loss of the corona is shown to have taken place twice within the Asclepiadeae alone, and other instances of loss are mentioned for this tribe. The findings are supported by those of Liede (2001). Based on the phylogenetic reconstruction presented here, it is most probable that pollinia in the Periplocoideae have arisen independently from those in the higher asclepiads. In addition, there is support for two separate origins of pollinia within Periplocoideae, as proposed in Verhoeven and Venter's (2001) contribution.

The sixth paper, by Albers and Meve (2001), is a karyological survey of the traditional Asclepiadaceae, including chromosome numbers for 299 taxa published for the first time. The basic chromosome number of $x = 11$ was found in 96% of the taxa investigated, with no deviations from this number found in Periplocoideae or Secamonoideae. A general trend of decreasing size of karyotypes is described, with the largest karyotype lengths found in the basal groups and the smallest in the Asclepiadeae, which is presumed to be the most derived tribe in the Asclepiadoideae.

In the seventh paper, Liede (2001) assesses the subtribe Astephaninae (Asclepiadeae) based on *trn*T-L and *trn*L-F spacer and *trn*L intron sequences. The traditional delimitation of the tribe was based on the lack of a staminal corona. Twelve representatives of the Astephaninae, as traditionally circumscribed, were analyzed. The taxa were found to fall into two main clades. One of them, the Astephaninae clade, contains only the Old World genera *Astephanus* s. str., *Microloma*, and *Oncinema*. The New World taxa previously included in the genus *Astephanus*, in contrast, come out in the unrelated Metastelminae clade. The other main clade, the Tylophorinae clade, comprises seven former Astephaninae genera. This study shows that a corona has been lost at least twice within the taxa studied and underscores the danger of placing too much weight on any one character.

The papers presented here are a testimony to the progress made in our understanding of the phylogeny and evolution of the Apocynaceae s.l., but they also serve to highlight areas that are still most in need of further work. Foremost among these, at higher taxonomic levels, is the taxonomic position of the Periplocoideae. This subfamily is the key to determining whether or not the former Asclepiadaceae are monophyletic. Much work also remains to be done in elucidating phylogenetic relationships among the genera of the Periplocoideae. Molecular evidence would be a welcome addition to the morphological data available. Within the Rauvolfioideae and Apocynoideae the relationships among the tribes are still vague, and the placement and significance of taxa such as *Baissea* remain an enigma. Phylogenetic relationships between Apocynoideae of the Old World and those of the New World are uncharted waters. Within Asclepiadoideae, most work still remains to be done in Marsdenieae

and Asclepiadeae. In Marsdenieae, major difficulties still exist as to generic limits; in addition, the relationship of Marsdenieae and Ceropegieae remains uncertain. In the large tribe Asclepiadeae, much work remains to be done at all taxonomic levels: work on the composition and delimitation of subtribes is desirable, and there are large, presumably heterogeneous genera that need to be sorted out. For a student interested in working on the systematics of a fascinating group and not afraid of complexity, many opportunities await.

Literature Cited

Albers, F. & U. Meve. 2001. A karyological survey of Asclepiadoideae, Periplocoideae, and Secamonoideae, and evolutionary considerations within Apocynaceae s.l. Ann. Missouri Bot. Gard. 88: 624–656.

Civeyrel, L. & N. Rowe. 2001. Phylogenetic relationships of Secamonoideae based on the plastid gene *mat*K, morphology, and biomechanics. Ann. Missouri Bot. Gard. 88: 583–602.

———, A. Le Thomas, K. Ferguson & M. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *mat*K sequences. Molec. Phylogenet. Evol. 9: 517–527.

Demeter, K. 1922. Vergleichende Asclepiadeenstudien. Flora 115: 130–176.

Endress, M. E. 2001. Apocynaceae and Asclepiadaceae: United they stand. Haseltonia 8: (in press).

——— & P. Bruyns. 2000. A revised classification of the Apocynaceae s.l. Bot. Rev. (Lancaster) 66: 1–56.

———, B. Sennblad, S. Nilsson, L. Civeyrel, M. Chase, S. Huysmans, E. Grafström & B. Bremer. 1996. A phylogenetic analysis of Apocynaceae s. str. and some related taxa in Gentianales: A multidisciplinary approach. Opera Bot. Belg. 7: 59–102.

Fishbein, M. 2001. Evolutionary innovation and diversification in the flowers of Asclepiadaceae. Ann. Missouri Bot. Gard. 88: 603–623.

Goyder, D. 1999. The Asclepiadaceae—A figment of our imagination? Pp. 309–317 *in* J. Timberlake & S. Kativu (editors), African Plants: Biodiversity, Taxonomy and Uses. Royal Botanic Gardens, Kew.

Judd, W. S., R. W. Sanders & M. J. Donoghue. 1994. Angiosperm family pairs: Preliminary phylogenetic analyses. Harvard Pap. Bot. 1(5): 1–51.

Jussieu, A. L. de. 1789. Genera Plantarum. Herissant, Paris.

Kunze, H., U. Meve & S. Liede. 1994. *Cibirhiza albersiana*, a new species of Asclepiadaceae, and establishment of the tribe Fockeeae. Taxon 43: 367–376.

Leeuwenberg, A. J. M. 1994. Taxa of the Apocynaceae above the genus level. Series of revisions of Apocynaceae, XXXVIII. Wageningen Agric. Univ. Pap. 94(3): 45–60.

Liede, S. 2001. Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) reconsidered: New evidence based on cpDNA spacers. Ann. Missouri Bot. Gard. 88: 657–668.

——— & A. Täuber. 2000. *Sarcostemma* R. Br. (Apocynaceae–Asclepiadoideae)—A controversial generic circumscription reconsidered: Evidence from *trn*L-F spacers. Pl. Syst. Evol. 225: 133–140.

———, A. Täuber & J. Schneidt. Molecular considerations on the Tylophorinae K. Schum. (Apocynaceae–Asclepiadoideae). Edinburgh J. Bot. (in press).

Potgieter, K. & V. A. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.

Safwat, F. M. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. Ann. Missouri Bot. Gard. 49: 95–129.

Schick, B. 1982. Zur Morphologie, Entwicklung, Feinstruktur und Funktion des Translators von *Periploca* L. (Asclepiadaceae). Trop. Subtrop. Pflanzenwelt 40: 513–553.

Schumann, K. 1895. Apocynaceae and Asclepiadaceae. *In* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien 4(2): 109–306. Engelmann, Leipzig.

Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.

——— & ———. 2000. Is there a justification for differential a priori weighting in coding sequences? A case study from *rbc*L and Apocynaceae s.l. Syst. Biol. 49: 101–113.

———, M. E. Endress & B. Bremer. 1998. Morphology and molecular data in phylogenetic fraternity—The tribe Wrightieae (Apocynaceae) revisited. Amer. J. Bot. 85: 1143–1158.

Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996. The subfamilial and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.

Venter, H. J. T. & R. L. Verhoeven. 2001. Diversity and relationships within the Periplocoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 550–568.

Verhoeven, R. L. & H. J. T. Venter. 1998. Pollinium structure in Periplocoideae (Apocynaceae). Grana 37: 1–14.

——— & ———. 2001. Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 569–582.

Wanntorp, H.-E. 1988 [1989]. The genus *Microloma* (Asclepiadaceae). Opera Bot. 98: 1–69.

PHYLOGENETIC RELATIONSHIPS WITHIN APOCYNACEAE S.L. BASED ON *trn*L INTRON AND *trn*L-F SPACER SEQUENCES AND PROPAGULE CHARACTERS[1]

Kurt Potgieter[2] and Victor A. Albert[3]

ABSTRACT

Relationships within Apocynaceae s.l. (Apocynaceae s. str., Periplocaceae, and Asclepiadaceae) were investigated by maximum parsimony analysis of morphological and molecular sequence data. Sequences of the plastid *trn*L intron and *trn*L-F spacer for 152 accessions for representatives of all major tribes were included in this study; 96% of these sequences represent new data. Two outgroups were selected from the closely related Loganiaceae. The total evidence matrix incorporated *trn*L intron and *trn*L-F spacer sequences, insertion/deletion information, and propagule characters. The phylogenetic hypothesis derived from this data set was used to evaluate the most current classification systems and was also used to investigate trends in seed dispersal. Apocynaceae s.l. are a strongly defined monophyletic group, a finding that should be reflected in taxonomic treatments. The recognition of three of the five subfamilies proposed by Endress and Bruyns in 2000 is supported by the monophyletic Periplocoideae, Secamonoideae, and Asclepiadoideae, whereas the paraphyletic Rauvolfioideae and Apocynoideae are not supported. The precise position of the Periplocoideae, however, remains unclear. Traditional tribal delimitations were less congruent with our total evidence phylogeny. The evolution of seed comas and enhanced long-distance dispersal within the Apocynaceae s.l. probably contributed to accelerated cladogenesis, ultimately giving rise to the majority of the extant genera within the family. Phylogeographic analysis of our data provides some evidence for a Gondwanan origin of the family.

Key words: Apocynaceae, Asclepiadaceae, Gentianales, Periplocaceae, *trn*L intron, *trn*L-F spacer.

The Apocynaceae Jussieu (1789) are a large and distinct clade of the Gentianales with nearly 4800 species distributed among 480 genera (Mabberley, 1997; Struwe et al., 1994, 1998). The above numbers represent Apocynaceae sensu lato, which includes the previously recognized families Asclepiadaceae and Periplocaceae (Demeter, 1922; Safwat, 1962; Stevens, 1975; Struwe et al., 1994; Judd et al., 1994; Thorne, 1992; Struwe et al., 1998; Civeyrel et al., 1998; Potgieter & Albert, 1997, 1998a, b; Endress & Bruyns, 2000). The Asclepiadaceae and Periplocaceae represent clades showing parallel phylogenetic trends toward reproductive synorganization (Sennblad & Bremer, 1996; Civeyrel et al., 1998). Following the work of Brown (1810) and Schlechter (1905), the morphologically complex Asclepiadaceae and Periplocaceae have, until recently, been separately maintained. There has been recent interest in clarifying the phylogenetic relationships of the Apocynaceae, Asclepiadaceae,

[1] This research was supported by the Lewis B. & Dorothy Cullman Program for Molecular Systematics Studies, The New York Botanical Garden. We thank the following persons and institutions for their generous aid and support: A. Tull, University of Georgia at Athens, U.S.A.; M. Endress, University of Zürich, Switzerland; E. DeMarie, W. Cahilly, D. Daly, S. Mori, L. Struwe, and W. Thomas, New York Botanical Garden, U.S.A.; O. Seberg and B. Johansen, University of Copenhagen, Denmark; C. Morse, University of Connecticut at Storrs, U.S.A.; E. Gilding, Hawaii, U.S.A.; J. Miller, P. Berry, P. Lowry, A. Jardim, W. D. Stevens, Missouri Botanical Garden, U.S.A.; G. Plunkett, Virginia Commonwealth University, U.S.A.; D. Cárdenas L., Instituto Amazônico de Investigaciones Científicas SINCHI (COAH), Colombia; E. Zagory, University of California at Davis, U.S.A.; V. Leyman, F. Billiet, J. Rammelso, National Botanical Garden of Belgium, Belgium; J. Fisher, D. Bar-zi, S. Zona, Fairchild Tropical Garden, U.S.A.; and J. Kramer, University of Illinois at Urbana-Champaign, U.S.A. Special thanks to P. Berry and M. Endress, who provided encouragement at every step of the way. Also, thanks to collaborators of M. Endress for providing material: P. de Block, P. Forster, R. Gilbertson, M. Grosjean, B. Haber, P. Jenkins, D. Lorence, R. Moraes de Jesus, S. Perlman, H. Petignat, A. Sloth, and B. Smith. We appreciate useful discussion with J. Zarucchi and W. D. Stevens on morphological propagule characters. Outgroup taxa were sequenced at NYBG and at AMNH by J. Rova, Department of Systematic Botany, Gothenburg University, Sweden. *Tylophora* and *Secamone* sequences were kindly provided by L. Struwe to complete the survey of the family, for which we are extremely grateful. We also thank J. S. Farris, Naturhistoriska Riksmuseet, Stockholm, Sweden, for permission to initially use his unpublished equally weighted parsimony jackknife computer application, XAC. Thanks also to D. Swofford, Smithsonian Institution, Washington, for the use of PAUP 4.0.0d64.

[2] Department of Integrative & Molecular Physiology, University of Illinois, Urbana, Illinois 60801, U.S.A.

[3] Department of Biological Sciences, The University of Alabama, Tuscaloosa, Alabama 35401, U.S.A.

and Periplocaceae (Judd et al., 1994; Civeyrel, 1996; Endress et al., 1996; Sennblad & Bremer, 1996; Sennblad et al., 1998), but in most of these studies many relevant taxa were not considered, and thus the monophyly of the Apocynaceae s.l. has not been adequately tested. The only comprehensive survey published to date that has included at least one taxon from all recognized tribes of the Apocynaceae s.l. is the doctoral thesis of Sennblad (1997) and the paper based thereon (Sennblad & Bremer, 2000). In their analysis, however, support values for many clades were very low, so that relationships among clades were poorly resolved. Moreover, the precise placements of the Asclepiadaceae and Periplocaceae clades are in conflict among existing phylogenetic trees. The phylogenetic reconstruction presented here includes representatives of all recognized tribes of the entire Apocynaceae clade, and with 152 taxa, is nearly double the number of taxa included in the Sennblad and Bremer (2000) analysis. In addition, our analysis is based on molecular as well as morphological data. Therefore it represents the most comprehensive phylogenetic investigation published to date on Apocynaceae s.l.

Our cladistic analysis of the Apocynaceae s.l. uses both molecular (*trn*L intron and *trn*L-F spacer) sequence and morphological propagule character data to estimate phylogenetic relationships within the family. One hundred and fifty-two accessions (113 genera, 146 species), representing all the major infrafamilial groups, are represented. Six morphological characters related to seed dispersal were combined with the molecular matrix. These morphological characters were included in order to generate hypotheses of character evolution and to examine how propagule-related features correlate with Asclepiadaceae and Periplocaceae clade diversification. A brief cladistic biogeographic interpretation of this group is finally presented.

Materials and Methods

Species sampled and sources of plant material

Plant material used for DNA extraction was obtained from field, botanical garden, and herbarium sources (Appendix 1). Field- and garden-collected specimens were dried and stored in silica gel prior to DNA isolation. From herbarium specimens, ca. 0.5 cm^2 of plant tissue was removed and either stored in plastic tubes or used immediately. Plant parts used included leaves, flowers, and seeds.

DNA isolation, sequencing, and alignment

DNA was extracted according to two protocols: a silica milk method (Struwe et al., 1998) and a modified potassium ethyl xanthogenate (PEX)/urea procedure (after Jhingan, 1992). Both methods remove secondary compounds thought to interfere with PCR amplification. The silica milk extraction method is basically a scaled down version of the standard CTAB (hexadecyltrimethylammonium bromide) DNA extraction method (Doyle & Doyle, 1987), but the final step in DNA isolation and purification is based on DNA: silica affinity (Boom et al., 1990; Savolainen et al., 1995) and not precipitation.

The *trn*L-F region of the plastid genome was amplified using standard PCR parameters according to Taberlet et al. (1991). Bovine serum albumin (BSA, 0.04%) was included in the PCR recipe to facilitate the likelihood of PCR product. The PCR primers correspond to the forward "c" (CGAAATCGGTAGACGCTACG) and "e" (GGTTCAAGTCCCTCTATCCC), and the reverse "d" (GGGGATAGAGGGACTTGAAC) and "f" (ATTTGAACTGGTGACACGAG) primers (Taberlet et al., 1991). Every attempt was made to amplify the entire *trn*L-F region using primers "c" and "f," but when this was not possible, separate "c-d" and "e-f" amplifications were conducted. Amplification products were purified in a preparatory low-melt agarose gel (Fisher Biotech) and re-amplified prior to sequencing reactions. Cycle sequencing was performed with either fluorochrome-labeled or dRhodamine dideoxy chain terminators. The cycle sequencing products were cleaned using Princeton Separations Centri-Sep 100 columns (www.bioresearchonline.com, following the manufacturer's protocol) and evaluated with a Perkin Elmer Applied Biosystems ABI 377 automated sequencer and software. Sequence alignments for phylogenetic analysis were initially done in Sequencher 3.0 followed by visual estimation.

Forty-eight binary insertion/deletion characters were assigned to account for structural (insertion/deletion) information (Table 1). The *trn*L (UAA) 3′ and ambiguous regions of sequence were excluded from maximum parsimony analysis and from the global alignment of 154 sequences (available on request).

Morphological data

Fruit and seed characters related to dispersal mode (Table 2) were obtained from primary literature sources (denoted by * in the reference section),

Table 1. Sequence insertion/deletion characters that were scored from the total Apocynaceae s.l. *trn*L intron and *trn*L-F spacer alignment (available on request). The indel # lists the number and the range provides the exact location of the gap in the final alignment for the contiguous intron and spacer.

*trn*L intron				*trn*L-F spacer			
Indel #	Range (bp)	Indel #	Range (bp)	Indel #	Range (bp)	Indel #	Range (bp)
1	73 & 88	17	307–310	30	667–672	46	1049–1057
2	122–126/141–144	18	311–314	31	706–722	47	1114–1116
3	130–140	19	382–470	32	731–739	48	1189–1194
4	127–140	20	299–300	33	786–844		
5	135–140	21	301–302	34	824–1219		
6	134–140	22	293–302	35	873–878		
7	133–140	23	321–327	36	819		
8	152–157	24	344–367	37	838–843		
9	165	25	354–359	38	883–1044		
10	235–241	26	388	39	923–936		
11	233–253	27	425 -138	40	924–936		
12	281–286	28	477–485	41	921–936		
13	291–300	29	500	42	982–991		
14	505–507			43	986–994		
15	531			44	1026–1047		
16	564–570			45	1053–1078		

as well as from personal observations. Interpretation of character states taken from the literature and/or from herbarium material was problematic given the state of the material and the lack of comprehensive propagule studies of the group as a whole. Since a large number of sources were consulted, character definitions were not consistent among these authors. Our morphological data set was, however, verified by consultation with M. Endress, J. Zarucchi, and W. D. Stevens. Definitions of characters and their states largely follow Endress et al. (1996).

PHYLOGENETIC ANALYSIS

The molecular data set and the combined data matrix (available upon request) were subjected to phylogenetic analysis. The molecular matrix consisted of the *trn*L intron, *trn*L-F spacer nucleotide sequence (see Table 3 for sequence characteristics), and binary insertion/deletion characters (Table 1). The combined matrix, however, consisted of both the molecular and the morphological propagule matrices so that considerations of morphological evolution could be made within the context of all available data (see Luckow & Bruneau, 1997). Each matrix was analyzed using Fitch parsimony (Fitch, 1971) as implemented in PAUP 4.0.0d64 (Swofford, 1998). The following options were used to find the shortest trees that were spread over several disjunct optima or islands: 100 replicates of random sequence addition order, saving no more than 3 trees longer than an arbitrarily low "dummy" tree length per replicate, MULPARS activated, and TBR branch swapping. Following this initial search, the shortest trees obtained were amplified in number by swapping on trees in memory (using Nearest-Neighbor Interchange or NNI branch swapping) until a limit of 1000 trees was reached; only most parsimonious trees were saved. These tree amplifications were performed in order to approximate asymptotically all possible non-polytomous resolutions permitted by the data (Sanderson & Doyle, 1993). Strict consensus trees of the NNI-swapped trees were constructed to summarize components shared among all primary trees. Support for particular groups was evaluated using parsimony jackknife without character weights (Farris et al., 1996). One thousand jackknife replicates ("emulate Jac" on) with branch swapping (and 5 random addition orders per replicate) were performed using PAUP. Only groups with jackknife frequency of 50% or greater are considered to have support (see Farris et al., 1996); 63% or higher parsimony jackknife frequencies represent the equivalent of one uncontradicted synapomorphy (Farris et al., 1996; Struwe et al., 1998). Character evolution was studied with MacClade 3.0 (Maddison & Maddison, 1992). Character optimizations on strict consensus trees were made using the "hard polytomy" option, which assumes that every branch of a polytomy has acquired a character state independently from the others. For counts of numbers of particular char-

Table 2. Propagule character matrix for genera within Apocynaceae s.l. Propagule and geographic character optimization was performed but is not provided. Geographic distribution pertains to species or genera where applicable: this character was, however, not included in the actual phylogenetic analysis but is provided here for completeness.

	Exocarp	Mesocarp	Pericarp sclerification	Seed presentation	Seed margin	Seed coma	Distribution
Bonyunia superba	0	0	2	0	1	1	0
Strychnos tomentosa	0	1	0	0&2	1&2	1	0
Acokanthera oppositifolia	0	1	0	2	2	1	1
Acokanthera rotundata	0	1	0	2	2	1	1
Allamanda	0	0	0	0	1	1	0
Alstonia boonei	0	0	0	0	2	0	1
Alyxia buxifolia	0	1	2	0	2	1	3
Amsonia orientalis	0	0	0	0	0&2	1	5
Amsonia tabernaemontana	0	0	0	0	2	1	6
Apocynum androsaemifolium	0	0	0	0	2	2	6
Apocynum cannabinum	0	0	0	0	2	2	6
Asclepias curassavica	0	0	0	0	1	2	0&7
Asclepias syriaca	0	0	0	0	1	2	6
Asclepias tuberosa	0	0	0	0	1	2	6
Aspidosperma australe	0	0	1&2	0	1	1	0
Aspidosperma cuspa	0	0	1&2	0	0	1	0
Aspidosperma desmanthum	0	0	1&2	0	1	1	0
Aspidosperma megalocarpon	0	0&1	2	0	1	1	0
Aspidosperma myristicifolium	0	0	1&2	0	1	1	0
Aspidosperma pyrifolium	0	0	1&2	0	1	1	0
Aspidosperma quebracho-blanco	0	0	1&2	0	1	1	0
Baissea leonensis	0	0	0	0	2	2	1
Beaumontia grandiflora	0	0	1&2	0	2	2	0&2&4
Bonafousia siphilitica	0	1	0	1	2	1	0
Callichilia barteri	0	1	0	1	2	1	1
Calotropis procera	0	0	0	0	1	2	2&5
Caralluma crenulata	0	0	0	0	1	2	1
Caralluma gerstneri	0	0	0	0	1	2	1
Carissa bispinosa	0	1	0	2	2	1	1
Carissa carandas	0	1	0	2	2	1	2
Catharanthus roseus	0	0	0	0	2	1	2
Cerbera manghas	1	0	1&2	0	0	1	3&4
Cerbera odollam	1	0	1&2	0	0	1	4
Cerberiopsis candelabra	1	0	1&2	0	0	1	4
Ceropegia sandersoni	0	0	0	0	1	2	1
Chonemorpha fragrans	0	0	0	0	2	2	2&3
Condylocarpon amazonicum	0	0	2	0	2	1	0
Condylocarpon sp.	0	0	2	0	2	1	0
Couma macrocarpa	0	1	0	2	2	1	0
Cryptolepis oblongifolia	0	0	0	0	2	2	1
Cryptostegia grandiflora	0	0	1&2	0	2	2	2
Cycladenia humilis	0	0	0	0	2	2	6
Cynanchum sp.	0	0	0	0	1	2	1&2&3&4&5&6
Dischidia bengalensis	0	0	0	0	1	2	4&5
Ditassa sp.	0	0	0	0	1	2	0
Dregea sinensis	0	0	1&2	0	1	2	5
Duvalia elegans	0	0	0	0	1	2	1
Duvaliandra dioscoridis	0	0	0	0	1	2	1
Echidnopsis cereiformis	0	0	0	0	1	2	1
Echidnopsis dammanniana	0	0	0	0	1	2	1
Echites umbellata	0	0	0	0	2	2	0&6&7
Fockea cylindrica	0	0	0	0	1	2	1
Fockea edulis	0	0	0	0	1	2	1

Table 2. Continued.

	Exocarp	Mesocarp	Pericarp sclerification	Seed presentation	Seed margin	Seed coma	Distribution
Fockea sinuata	0	0	0	0	1	2	1
Forsteronia corymbosa	0	0	0	0	2	2	7
Frerea indica	0	0	0	0	1	2	2
Funtumia elastica	0	0	1&2	0	2	2	1
Galactophora crassifolia	0	0	0	0	2	2	0
Geissospermum laeve	0	1	0	2	2	1	0
Gomphocarpus physocarpus	0	0	0	0	2	2	1
Gomphocarpus sp.	0	0	0	0	2	2	1
Gonolobus patens	0	0	0	0	1	2	0&6
Haplophyton crooksii	0	0	0	0	2	2	0&6
Himatanthus sp.	0	0	0	0	1	1	0
Himatanthus tarapotensis	0	0	0	0	1	1	0
Holarrhena pubescens	0	0	0	0	2	2	1&2
Hoya australis	0	0	0	0	2	2	3&4
Huernia keniensis	0	0	0	0	1	2	1
Hunteria umbellata	0	1	0	2	2	1	1
Isonema smeathmanni	0	0	0	0	2	2	1
Lacmellea aculeata	0	1	0	2	2	1	0
Landolphia incerta	0	1	0	2	2	1	1
Laxoplumeria baehniana	0	0	0	0	2	0	0
Lepiniopsis ternatensis	0	1	2	0	2	1	3&4
Macoubea guianensis	0	1	0	1	2	1	0
Mandevilla sp.	0	0	0	0	2	2	0
Marsdenia amorimii	0	0	0	0	1	2	0
Mascarenhasia arborescens	0	0	0	0	2	2	2
Matelea sp.	0	0	0	0	2	2	0&6
Melodinus monogynus	0	1	0	2	2	1	4
Micholitzia obcordata	0	0	0	0	1	2	2&4
Microplumeria anomala	0	0	0	0	2	1	0
Molongum laxum	0	1	0	2	2	1	0
Mondia whiteii	0	0	0	0	1	2	1
Neisosperma nakiana	0	1	2	0	0	1	4
Nerium oleander	0	0	0	0	2	2	1&2&5
Ochrosia elliptica	0	1	2	0	0	1	3&4
Ophionella arcuata	0	0	0	0	1	2	1
Orbea variegata	0	0	0	0	1	2	1
Orbeanthus hardyi	0	0	0	0	1	2	1
Oxystelma bornouense	0	0	0	0	2	2	1
Pachycarpus sp.	0	0	0	0	1	2	1
Pacouria guianensis	0	1	0	2	2	1	0
Parsonsia eucalyptophylla	0	0	0	0	2	2	3
Peltastes peltatus	0	0	0	0	2	2	0
Pentalinon luteum	0	0	0	0	2	2	7
Periploca graeca	0	0	0	0	2	2	5
Petopentia natalensis	0	0	0	0	2	2	1
Philibertia gilliesii	0	0	0	0	1	2	0
Piaranthus geminatus	0	0	0	0	1	2	1
Picralima nitida	0	1	0	2	2	1	1
Plectaneia stenophylla	0	0	0	0	0	1	2
Pleiocarpa mutica	0	1	0	2	2	1	1
Plumeria alba	0	0	0	0	0	1	7
Plumeria obtusa	0	0	0	0	0	1	0&7
Prestonia mollis	0	0	0	0	2	2	0
Prestonia quinquangularis	0	0	0	0	2	2	0&7
Pteralyxia kauaiensis	0	1	2	0	2	1	1

Table 2. Continued.

	Exocarp	Mesocarp	Pericarp sclerification	Seed presentation	Seed margin	Seed coma	Distribution
Rauvolfia balansae	0	1	2	0	2	1	4
Rauvolfia serpentina	0	1	2	0	2	1	2&4
Saba comorensis	0	1	0	2	2	1	1&2
Sarcostemma mulanjense	0	0	0	0	1	2	1&2
Sarcostemma stolonifera	0	0	0	0	1	2	1&2
Secamone glaberrima	0	0	0	0	2	2	2
Secamone oleaefolia	0	0	0	0	2	2	2
Skytanthus acutus	0	0	1&2	0	2	1	0
Stapelia leendertziae	0	0	0	0	1	2	1
Stapelia peglere	0	0	0	0	1	2	1
Stapelia revoluta	0	0	0	0	1	2	1
Stapelia schinzii	0	0	0	0	1	2	1
Stomatostemma monteiroae	0	0	0	0	2	2	1
Strempeliopsis strempelioides	0	0	0	0	0	1	7
Strophanthus gratus	0	0	1&2	0	2	2	1
Tabernaemontana citrifolia	0	1	0	1	2	1	7
Tabernaemontana floribunda	0	1	0	1	2	1	3&4
Tabernaemontana sp.	0	1	0	1	2	1	2&4&7
Tabernanthe iboga	0	1	0	1	2	1	1
Tavaresia grandiflora	0	0	0	0	1	2	1
Telosma cordata	0	0	1&2	0	1	2	4
Thevetia ahouai	0	1	2	0	0	1	0
Thevetia peruviana	1	0	1&2	0	0	1	0
Tonduzia longifolia	0	0	0	0	0	0	0
Tonduzia stenophylla	0	0	0	0	0	0	0
Trachelospermum jasminoides	0	0	0	0	2	2	4&5
Trichocaulon triebneri	0	0	0	0	1	2	1
Tridentea longipes	0	0	0	0	1	2	1
Tromotriche revoluta	0	0	0	0	1	2	1
Tweedia coerulea	0	0	0	0	1	2	0
Tylophora sylvatica	0	0	0	0	1	2	1&2
Urceola rosea	0	0	0	0	2	2	2&4
Vallesia antillana	0	1	2	0	2	1	6&7
Vinca difformis	0	0	0	0	2	1	5
Vinca minor	0	0	0	0	2	1	5
Vincetoxicum hirundinaria	0	0	0	0	1	2	5
Vincetoxicum nigrum	0	0	0	0	1	2	5
Voacanga africana	0	1	0	1	2	1	1
Wrightia tomentosa	0	0	0	0	2	2	2&1

Characters & States

Exocarp
0: thin
1: thick

Mesocarp
0: dry
1: fleshy

Pericarp sclerification
0: non-sclerified
1: mesocarp
2: endocarp

Seed presentation
0: naked
1: arillate
2: embedded in pulp

Seed margin
0: not circumalate
1: circumalate
2: naked

Seed coma
0: ill defined
1: absent
2: well defined

Distribution
0: South–Meso America
1: Africa
2: Indian Ocean Basin
3: Australia–New Guinea
4: Pacific
5: temperate Eurasia
6: North America
7: Caribbean

Table 3. Sequence characteristics of the plastic *trn*L intron and *trn*L-F spacer sequence for members of the Apocynaceae s.l.

Sequence characteristic	*trn*L intron	*trn*L-F spacer
Nucleotide sites (bp)		
Total length (without gaps)	range (348–496) mean = 481	range (246–378) mean = 322
No. aligned positions (with gaps)	625	610
No. (%) ambiguous	58 (9.3)	164 (26.9)
No. (%) constant	319 (51)	214 (35.1)
No. (%) autapomorphic	95 (15.2)	75 (12.3)
No. (%) phylogenetically informative	153 (24.5)	157 (25.7)
Length variation (bp)		
No. of unambiguous alignment gaps	51	52
No. (size range) of deletions	13 (1–89)	11 (6–396)
No. (size range) of insertions	16 (1–21)	8 (1–14)
No. of gaps phylogenetically informative	29	19
No. (%) of point gaps	13224 (15.1)	19000 (27.7)
Sequence divergence (%)	0.2–12.2	0–17.6
G + C (%) content (range, mean)	range (35.9–39.9) mean = 36.9	range (33.9–37.8) mean = 35.3
Ts/Tv ratio (range)	1.27–1.28	1.07–1.08

acter state transformations, only unambiguous events were included (excluding outgroup taxa).

Results and Discussion

Molecular and combined molecular–morphological phylogeny

Parsimony analysis of the molecular and combined matrix yielded **132**/120 most parsimonious trees of length **1218**/1294 steps (including autapomorphies and uninformative characters) and ensemble consistency (CI) and retention (RI) indices (Farris, 1989) of **0.53**/0.52 and **0.89**/0.89, respectively. A strict consensus was computed after the **132**/120 initial trees were amplified to a maximum of 1000 (Fig. 1). Values that pertain to the analysis of the molecular data (number of trees, CIs, RIs, jackknife support) always appear first in bold; those that apply to analysis of the combined data follow.

The phylogenies derived from the molecular and combined data are similar in both strict consensus structure and jackknife support. A single incongruency involves a weak association between *Rauvolfia* and *Tonduzia* (**55**/<50%). Other variations involve slight differences in tree resolution (Figs. 1, 2, indicated by dashed nodes)—the strict consensus of the molecular matrix is slightly less resolved than that for the combined analysis. The increased resolution obtained by combining the molecular and morphological data further supports the usefulness of including morphological characters in this analysis. As would be expected, the addition of the few representative morphological characters did not significantly influence parsimony jackknife support, except for the *Neisosperma/Ochrosia* clade where support increased from <**50** to 73%. The other sizable difference in support is seen at the very base of the tree for the genus *Aspidosperma* (<**50**/63). These large changes in support argue for the continued recognition of *Neisosperma* and *Ochrosia* and perhaps the re-examination of circumscriptions for these genera (see Leeuwenberg (1994) for difference of opinion, and Potgieter (1999) for the examination of the genus *Aspidosperma*).

Parsimony jackknife values demonstrate strong support for the basic internal structure of the tree. Notably, (1) the monophyly of Apocynaceae s.l. is supported by **100**/100% of the jackknife replicates: similar branching order was seen for the analysis of a small number (11) of representative members of Apocynaceae using *trn*L spacer sequences together with Gentianaceae and Rubiaceae as outgroups (Struwe et al., 1998); (2) the genus *Aspidosperma* and its allies are sister to the rest of Apocynaceae in **94**/96% of replicates; (3) the genus *Alstonia* s. str. (i.e., excluding *Tonduzia*), in turn, is the sister group to the rest of the family at **95**/97%; (4) the fleshy-fruited genera *Acokanthera*

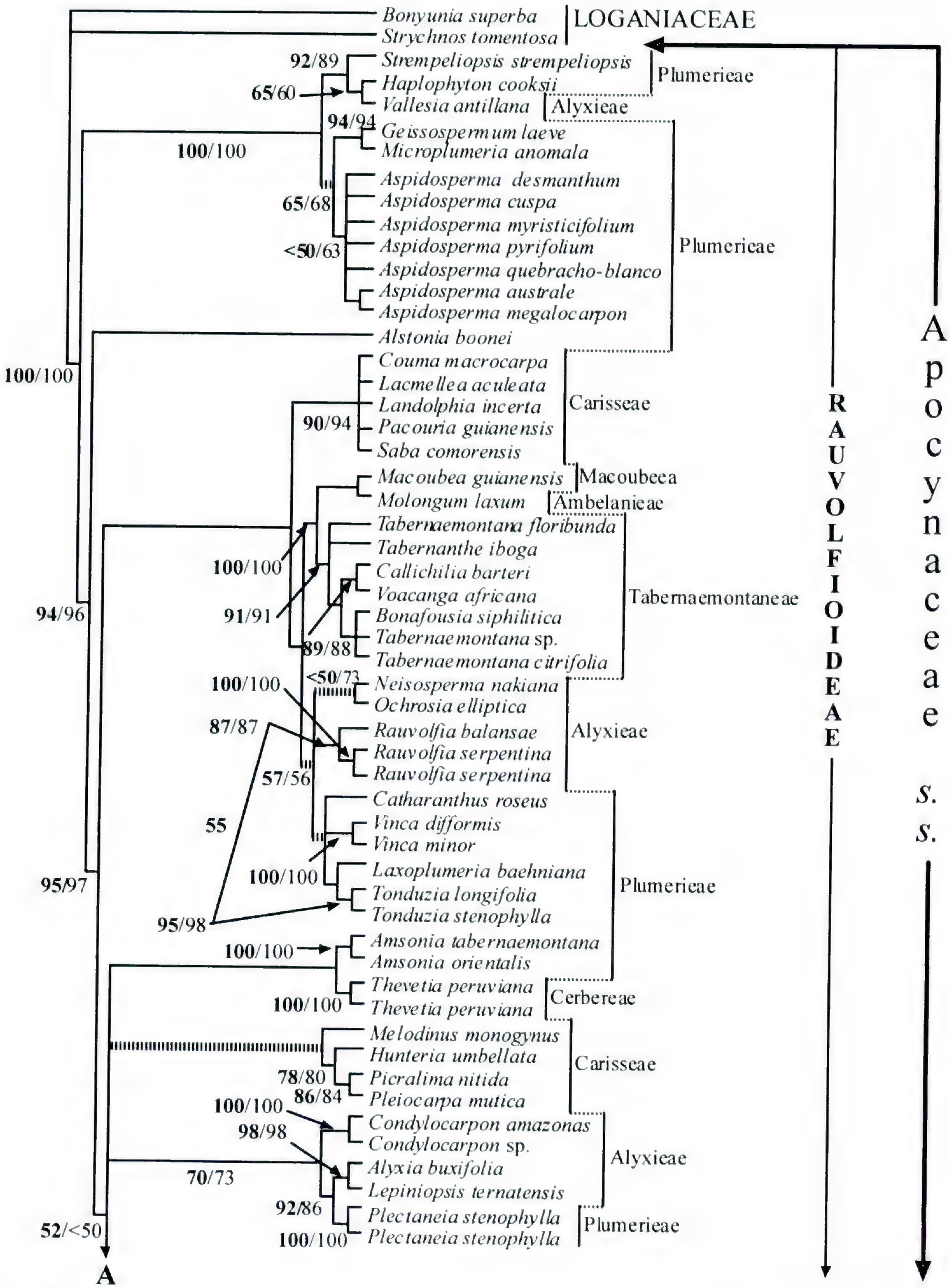
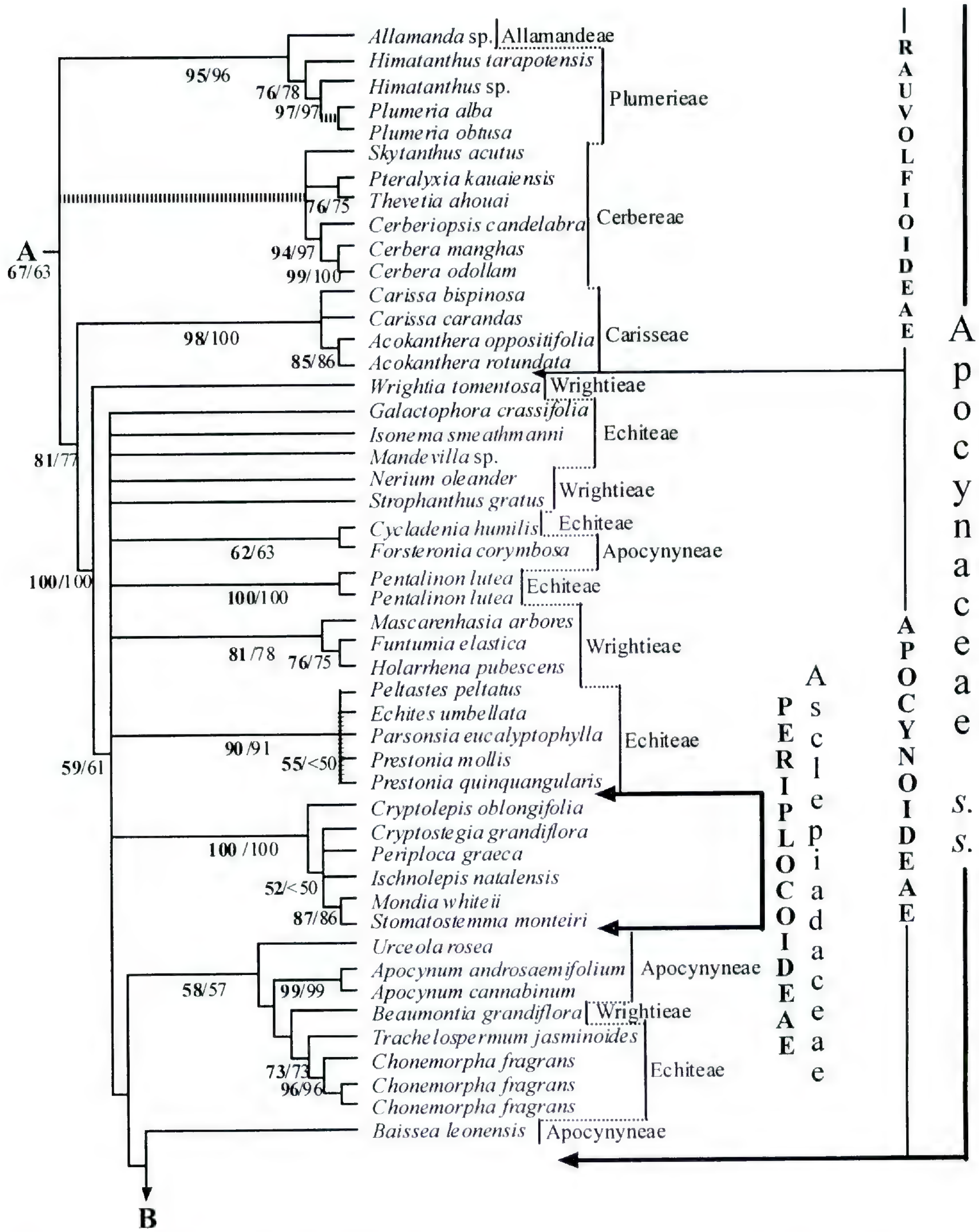


Figure 1 (pp. 530–532). Strict consensus of 1000 equally parsimonious trees (**1218**/1294 steps) for the molecular (*trn*L intron and *trn*L-F spacer nucleotide) matrix and the combined molecular and morphological (six propagule characters) data sets. The separate matrices are available from the first author upon request. Jackknife support values are indicated as the first value below the nodes for the tree generated from the molecular data alone—the second value represents jackknife support for the combined data matrix; black arrows denote support for a given node when space was limited. Dashed nodes are used to indicate additional structure seen in the combined data set, e.g., [(*Neisosperma*, *Ochrosia*), (*Catharanthus*–*Tonduzia*), (*Melodinus*–*Pleiocarpa*), (*Plumeria*), (*Skytanthus*–*Cerbera*), and the *Gomphocarpus*



clade], whereas dashed vertical lines indicate additional structure in the molecular data set [(*Peltastes–Prestonia*), (*Marsdenia, Telosma*)]. The only structural dissimilarity occurred in a relatively unsupported portion of the tree in the molecular clade consisting of [*Rauvolfia* (*Laxoplumeria, Tonduzia*)]. Consistency (CI) and retention (RI) indices for the molecular data set and the combined data set were **0.53**/0.52 and **0.89**/0.89, respectively. The subfamilial and tribal classifications sensu Leeuwenberg (1994) and Swarupanandan et al. (1996) are compared with this phylogenetic tree.

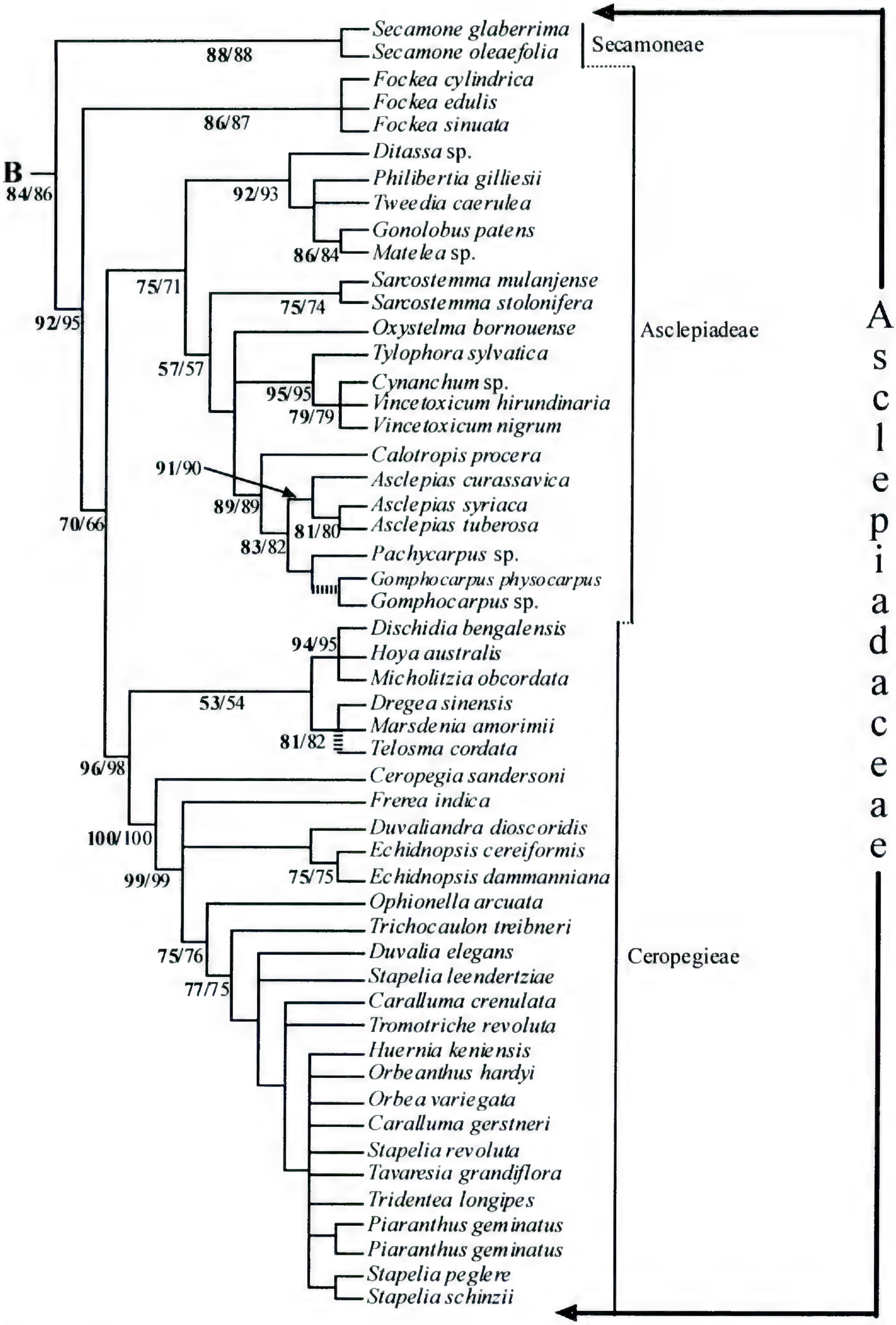


Figure 1. Continued.

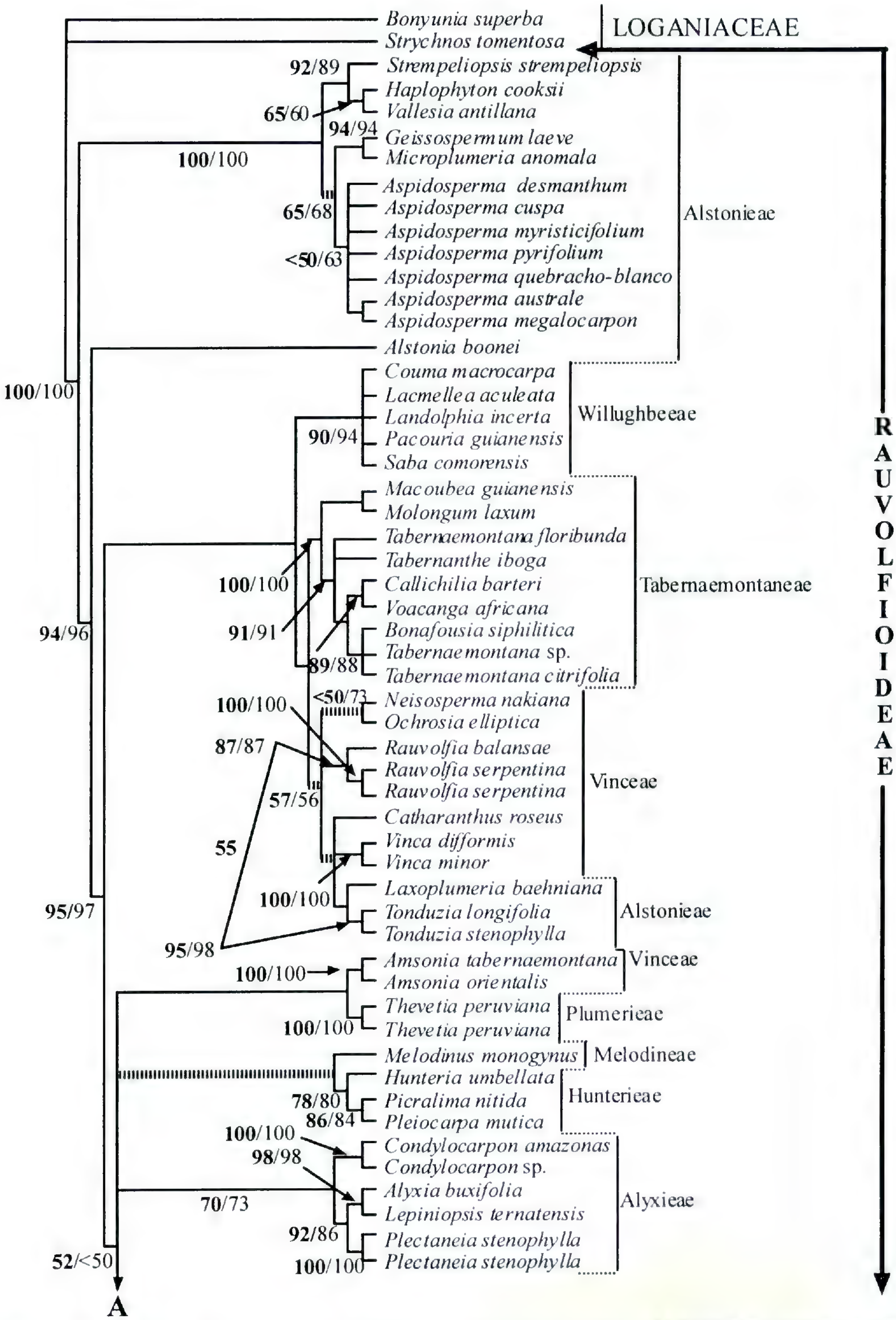
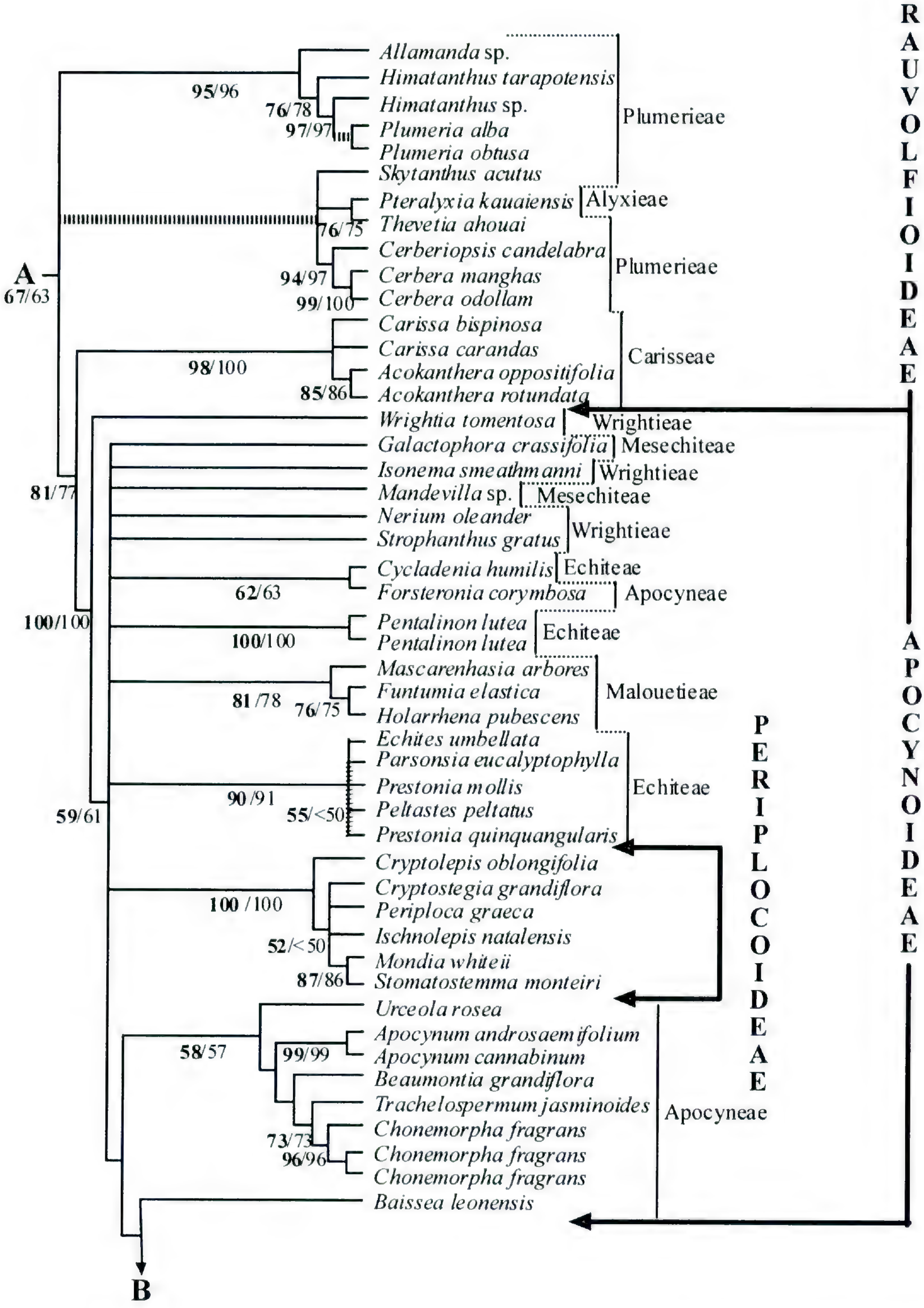


Figure 2 (pp. 533–535). Strict consensus of 1000 equally parsimonious trees (**1218**/1294 steps) for the molecular (*trn*L intron and *trn*L-F spacer nucleotide) matrix and the combined molecular and morphological (six propagule characters) data sets. The separate matrices are available from the first author upon request. Jackknife support values are indicated as the first value below the nodes for the tree generated from the molecular data alone—the second value represents jackknife support for the combined data matrix; black arrows denote support for a given node when space was limited. Dashed nodes are used to indicate additional structure seen in the combined data set, e.g., [(*Neisosperma*, *Ochrosia*), (*Catharanthus–Tonduzia*), (*Melodinus–Pleiocarpa*), (*Plumeria*), (*Skytanthus–Cerbera*), and the *Gomphocarpus*



clade] whereas dashed vertical lines indicate additional structure in the molecular data set [(*Peltastes–Prestonia*), (*Marsdenia, Telosma*)]. The only structural dissimilarity occurred in a relatively unsupported portion of the tree in the molecular clade consisting of [*Rauvolfia* (*Laxoplumeria, Tonduzia*)]. Consistency (CI) and retention (RI) indices for the molecular data set and the combined data set were **0.53**/0.52 and **0.89**/0.89, respectively. The subfamilial and tribal classification sensu Endress and Bruyns (2000) is compared with this phylogenetic tree.

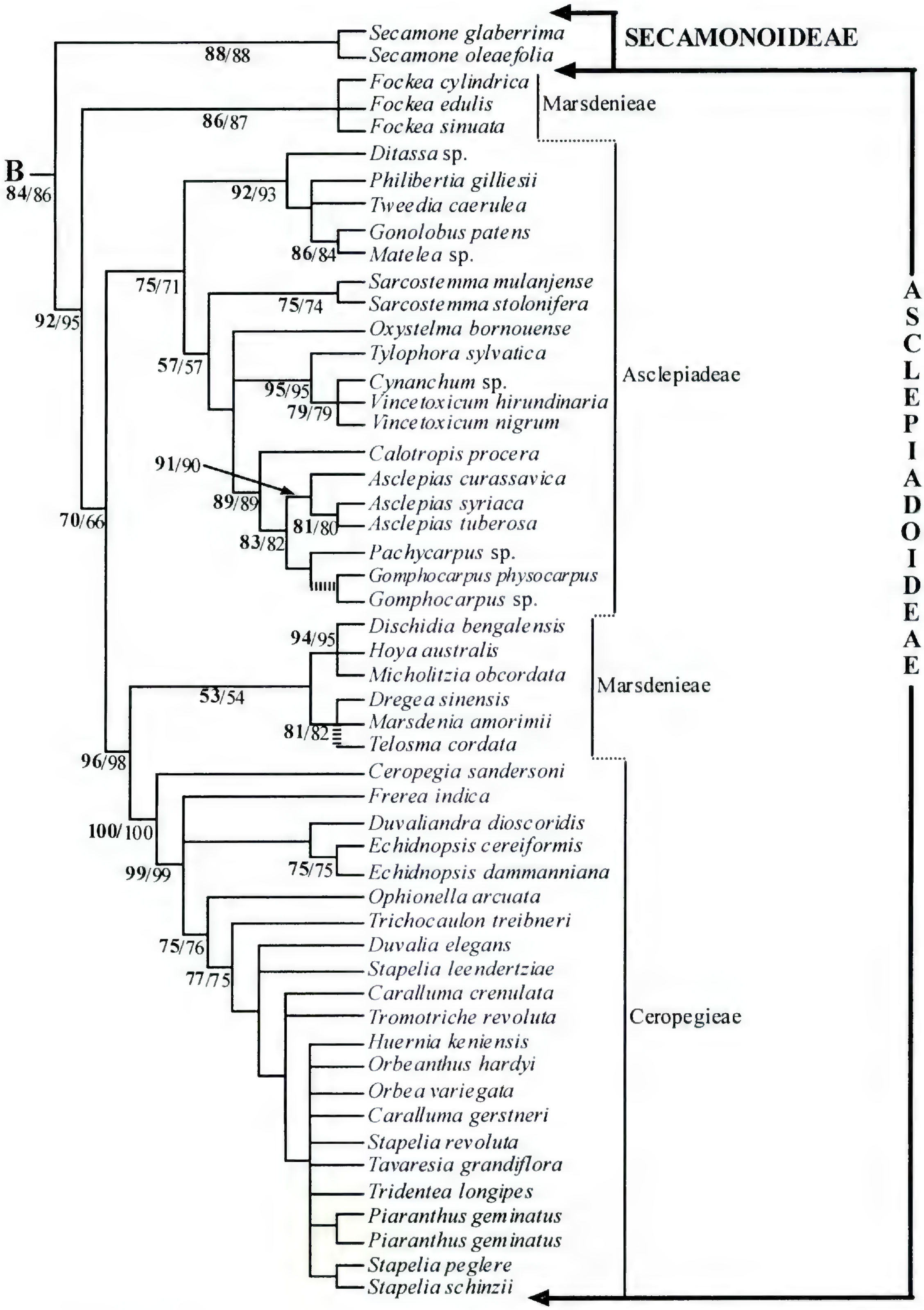


Figure 2. Continued.

and *Carissa* are resolved as sister genera to the "higher" Apocynaceae, Periplocaceae, and Asclepiadaceae in **81**/77% of replicates; and (5) the Apocynoideae, Periplocaceae, and Asclepiadaceae-clade is supported as monophyletic in **100**/100% of jackknife replicates, as are the Periplocaceae and Asclepiadaceae themselves (**100**/100% and **84**/86% of jackknife replicates, respectively).

THE APOCYNACEAE SENSU STRICTO ARE PARAPHYLETIC TO THE ASCLEPIADACEAE AND THE PERIPLOCACEAE

The non-monophyly of the traditionally maintained Apocynaceae s. str., with respect to the Periplocaceae and the Asclepiadaceae, is increasingly being recognized. For example, MacFarlane's (1933: 15) treatise on the evolution and distribution of both families included the explicit statement that "the extremely complex and highly evolved flower of Asclepiadaceae can be traced to have originated by graded progressive stages from the simpler ones of higher Apocynaceae." Recent cladistic analyses using morphological (Judd et al., 1994; Struwe et al., 1994) and molecular (Civeyrel, 1996; Civeyrel et al., 1998; Sennblad, 1997; Sennblad & Bremer, 1996, 2000; Sennblad et al., 1998) data have supported the contention that Asclepiadaceae and Periplocaceae are derived from within a larger, monophyletic Apocynaceae. The data presented here (Fig. 1) add further support to the naturalness of the Apocynaceae sensu lato by demonstrating that the Periplocaceae and Asclepiadaceae form monophyletic clades derived from within the higher Apocynaceae. We therefore recognize that the Apocynaceae s.l. consist of the subfamilies Periplocoideae, Secamonoideae, and Asclepiadaceae based on monophyly, but Apocynoideae and especially Rauvolfioideae are decidedly paraphyletic as delimited by Endress and Bruyns (2000). Our data therefore do not support recognition of Apocynoideae or Rauvolfioideae. Either the well-supported tribes can be maintained or many new subfamilies should be recognized (Sennblad, 1997; Civeyrel et al., 1998). We feel, however, that additional evidence is needed before such major classification changes are made. Sennblad and Bremer's (1996, 2000) phylogenetic analysis based on the *rbc*L gene suggested that Periplocaceae are most closely related to members of tribes Echiteae (*Prestonia* and *Mandevilla*) and Apocyneae (*Apocynum*). The Asclepiadaceae were, in turn, resolved as the sister group of this clade. In contrast, Civeyrel's (1996; Civeyrel et al., 1998) results, based on the *mat*K locus, showed the Periplocaceae and Asclepiadaceae to be sister taxa, with members of the Echiteae (*Prestonia*), Apocyneae (*Apocynum*), and Wrighteae (*Beaumontia*) forming a more basal clade. Both of these resolutions cannot be simultaneously correct, and, indeed, our analysis of characters from the *trn*L intron and the *trn*L-F spacer region (Fig. 1) echoes the need for further information to resolve this ambiguity.

SUBTRIBE CARISSINAE: THE SISTER GROUP TO HIGHER APOCYNACEAE

The condition of congenital syncarpy in tribe Carisseae sensu Leeuwenberg (1994) is polyphyletic (Fig. 1). The genus *Melodinus* (subtribe Melodininae) and the genera *Hunteria*, *Picralima*, and *Pleiocarpa* (subtribe Pleiocarpinae sensu Leeuwenberg, 1994; marginally monophyletic in **78**/80% of jackknife replicates) form part of a trichotomy at two nodes less derived (Fig. 1). *Acokanthera* and *Carissa* (subtribe Carissinae sensu Leeuwenberg, 1994) form a strong group (**98**/100%) that is supported (**81**/77%) as sister to the rest of the higher Apocynaceae (including the Periplocaceae and Asclepiadaceae). This is in agreement with findings of other recent molecular studies (Sennblad & Bremer, 1996; Endress et al., 1996; Civeyrel et al., 1998). Indeed, *Acokanthera* and *Carissa* were considered congeneric by Pichon (1948). These fleshy-fruited genera with plain style-heads and non-synorganized anthers have previously been considered among the most plesiomorphic taxa of "Carisseae" and, indeed, the Apocynaceae as a whole (Endress et al., 1996; Leeuwenberg, 1994). Phylogenetic evidence from *rbc*L and *mat*K data also refutes a basalmost position of the relatively unspecialized "Carisseae," but neither gene discerns the position of subtribe Carissinae as sister to the derived clades of the Apocynaceae s.l. (Civeyrel, 1996; Sennblad & Bremer, 1996).

*trn*L INTRON AND *trn*L-F SPACER COMPARISON TO RECENT PHYLOGENETIC IDEAS

The strict consensus tree from parsimony analysis of *trn*L intron and *trn*L-F spacer sequence and propagule characters for the Apocynaceae s.l. highlights discrepancies with the Endress and Bruyns (2000) hypothesis (Fig. 2). In this tree tribal delimitations in subfamily Rauvolfioideae sensu Endress and Bruyns (2000) are well supported, except for the following difference (Fig. 2, p. 533): the Alstonieae is polyphyletic—*Tonduzia* was grouped with *Alstonia* in tribe Alstonieae. In con-

trast, the genera *Tonduzia* and *Laxoplumeria* appear to be weakly associated with tribe Vinceae, which is in turn paraphyletic, with *Amsonia* grouping with *Thevetia peruviana* (Fig. 2, p. 533). Additionally, the Alyxieae appear to be polyphyletic: *Pteralyxia* (Alyxieae) is strongly associated with *Thevetia ahouai* (**76**/75%), which in turn is firmly nested within the Plumerieae (Fig. 2, p. 534). Note, however, that the genus *Thevetia* is polyphyletic in this phylogenetic tree; this could be interpreted as support for recognition of *Cascabela* as a genus distinct from *Thevetia*. These results require further study as the position of *T. peruviana* conforms to the expected position in studies by Sennblad and Bremer (1996) and Endress et al. (1996). Apocynoideae have very little resolution and, although weakly supported, the position of subfamily Periplocoideae appears to be nested within it (Fig. 2, p. 534). Subfamily Secamonoideae, represented by *Secamone*, is well supported (Fig. 2, p. 535), as is the Asclepiadoideae, the most highly derived subfamily (Fig. 2, p. 535). The tribe Marsdenieae is polyphyletic, with the genus *Fockea* representing the basalmost clade of the Asclepiadoideae and the *Dischidia, Hoya, Micholitzia, Dregea, Marsdenia, Telosma* clade sister to the Ceropegieae, the most derived tribe (Fig. 2, p. 535). Recognition of the tribe Fockeae (Kunze et al., 1994) is supported (Fig. 2, p. 535).

The remaining discussion largely follows the classification system sensu Endress and Bruyns (2000) as it is more congruent with our phylogenetic results (Fig. 2) than other current systems.

THE BASAL CLADES OF THE APOCYNACEAE

Analysis of both the molecular and the combined data matrix provides strong support for the basalmost clades of the Apocynaceae s.l. Previous molecular studies that used *rbc*L and *mat*K sequence (Civeyrel, 1996; Endress et al., 1996; Sennblad & Bremer, 1996; Civeyrel et al., 1998) identified the genus *Alstonia* s. str. as the most plesiomorphic lineage, but important taxa from the Plumerieae and Alyxieae, namely *Aspidosperma* and allies, were not included in their analyses. The results of this study show that a clade comprising *Aspidosperma, Geissospermum, Microplumeria, Haplophyton, Strempeliopsis*, and *Vallesia* is sister to the rest of the family (Fig. 2, p. 533; both the clade and its basal position are supported by **100**/100% and **94**/96% of jackknife replicates, respectively). These genera belong to the Alstonieae (Fig. 2, p. 533). In keeping with earlier molecular results, the next most basal taxon after this clade is *Alstonia* s. str. (Alstonieae; supported by **95**/97% of jackknife replicates). The genus *Tonduzia*, which has been synonymized with *Alstonia* (Pichon, 1947a; Gentry, 1983) and maintained in the latest monograph in *Alstonia* (Sidiyasa, 1998) was recognized by Endress and Bruyns (2000), who, however, placed both *Alstonia* and *Tonduzia*, as well as the poorly known genus *Laxoplumeria*, in tribe Alstonieae. In contrast, in the present phylogenetic study, there is no support for a close relationship between *Alstonia* and the latter two genera. Rather, both *Tonduzia* and *Laxoplumeria*—both from tropical America—appear to be weakly associated with tribe Vinceae, i.e., in a more derived position than the African and Asian *Alstonia* (Fig. 2). As noted above, the group traditionally held as most plesiomorphic, the Carisseae, is syncarpous, but is also highly derived (Fig. 2, p. 534). Members of tribes Carisseae and Alstonieae share relatively unspecialized flowers that have a uniformly receptive and secretory style-head and non-synorganized stamens. Species of *Haplophyton* and *Vallesia* have a vertically differentiated style-head, with basal stigmatic regions (characteristic of most Apocynaceae s.l.). Moreover, *Haplophyton* has stamens that are convergent over the style-head as do more derived members of the family, e.g., *Allamanda, Rauvolfia*, and most species of *Tabernaemontana* (Endress, 1986; Endress et al., 1996; Leeuwenberg, 1994; Pichon, 1950b; Potgieter, unpublished). *Alstonia* s. str., on the other hand, displays a vertically differentiated style-head without convergent stamens (Endress et al., 1996). Therefore, style-head specialization and the beginnings of synorganization appear to have evolved in parallel among lower clades of the Apocynaceae s.l., becoming fixed among the higher clades, but with a notable reversal in the tribe Carisseae. The molecular-derived relationships of the Apocynaceae s.l. to outgroup taxa remain unclear using both *trn*L intron and *trn*L-F spacer sequences (Struwe et al., 1998; pers. comm.) as well as data from the *mat*K and *rbc*L regions (Civeyrel, 1996; Civeyrel et al., 1998; Endress et al., 1996; Sennblad & Bremer, 1996). The Loganiaceae genera *Gardneria, Strychnos*, and *Usteria* appear to be morphologically similar in that they have flattened seeds with a central hilum (Leeuwenberg & Leenhouts, 1980; Struwe et al., 1994) in common with the genus *Aspidosperma* (Alstonieae).

EVOLUTION OF PROPAGULE CHARACTERS

Most examinations of character evolution in the Apocynaceae s.l. have centered on issues related

to floral evolution, i.e., synorganization and specialization of the androecial and gynoecial structures (e.g., Endress et al., 1996; Endress, 1994; Fallen, 1986; Judd et al., 1994; Kunze, 1990, 1993, 1996; Nilsson et al., 1993; Safwat, 1962). While floral complexity does increase with increasing phylogenetic derivation (Civeyrel, 1996; Endress et al., 1996; Sennblad & Bremer, 1996), associated traits (related to synorganization and specialization) are by no means fixed until the uppermost branches of the Apocynoideae (i.e., above the Carisseae) are reached. This is readily apparent when floral characters are optimized onto our molecular trees (unpublished results) and is clear from our discussion here of parallel trends of synorganization and specialization in the basalmost clades of Apocynaceae s.l. Indeed, with members of the Apocynaceae s.l. bearing the most complex flowers of any dicotyledonous angiosperm (Endress et al., 1983; Endress, 1994), to do full justice to the issue of floral character evolution will require many additional ontogenetic studies beyond those that have so far been performed.

Instead of focusing on these most popularly studied attributes of the Apocynaceae s.l., a subject that has not heretofore received such attention, the phylogenetic correlates of propagule characters, is presented. Several fruit and seed characteristics, likely correlated with dispersal mode, have been used in traditional classifications, but with the exception of syncarpy (Endress et al., 1996; Sennblad & Bremer, 1996), their phylogenetic and evolutionary significance has not yet been explored.

Most members of the Gentianales have syncarpous ovaries, but apocarpy is common among Apocynaceae s.l. and is the rule for Loganiaceae genera *Mitrasacme* and *Mitreola*, which are apically apocarpous (Conn & Brown, 1996; Endress et al., 1983; Fallen, 1983b, 1986; Struwe et al., 1994). Analysis of data from plastid genes, *mat*K, *rbc*L, and *trn*L-F (Civeyrel, 1996; Sennblad & Bremer, 1996; Civeyrel et al., 1998; Sennblad, 1997; Sennblad & Bremer, 2000; this paper, Fig. 2), indicates that apocarpy is the plesiomorphic state for the Apocynaceae s.l. (see above and Endress et al., 1996). In the Gentianaceae at least, congenital syncarpy is thought to be a prerequisite for the development of fleshy fruits (Struwe, 1999). In the Apocynaceae s.l., on the other hand, fleshy fruits are known among both congenitally syncarpous as well as apocarpous taxa. Fleshy fruits are often associated with animal-mediated dispersal syndromes, and are evaluated within a phylogenetic context.

Mesocarp is the differentiated ovary wall, excluding the inner and outer layers (endocarp and exocarp, respectively). Dry mesocarp is plesiomorphic for the Apocynaceae s.l., whereas fleshiness has been derived independently multiple times within this clade (Table 2). Fleshy mesocarp is known from the early-branching *Vallesia* and *Geissospermum* (Alstonieae), the relatively early-branching, syncarpous Willughbeeae (*Couma*, *Parahancornia*, *Saba*, *Lacmellea*, and *Landolphia*), the Tabernaemontaneae clade (most of which are apocarpous except for members of the former Ambelanieae), *Rauvolfia* (including both syncarpous and apocarpous species), *Neisosperma* and *Ochrosia* (Vinceae), the more derived and syncarpous genus *Melodinus* of the Melodineae, the apocarpous Hunterieae (*Hunteria*, *Picralima*, and *Pleiocarpa*), some syncarpous (*Lepiniopsis*) as well as apocarpous (*Alyxia*, *Pteralyxia*) Alyxieae, and *Cerbera* and *Thevetia* (Plumerieae), the syncarpous Carisseae (*Acokanthera* and *Carissa*) and *Thevetia ahouai*, which in this analysis, shows a weak relationship with *Amsonia* (Vinceae). Fleshy mesocarp, which has been used as a taxonomic character to unify Carisseae in traditional classifications (e.g., Leeuwenberg, 1994), is clearly inconsistent with the phylogenetic placement of the various syncarpous clades assigned to that tribe. Fleshiness probably correlates more with ecological traits, e.g., dispersal syndrome, than with anything else.

Endocarp in the Apocynaceae is primitively non-sclerified (Table 2). Sclerification of the endocarp is often associated with drupe development. Sclerified endocarp appears to have been derived in the Apocynaceae s.l. six times independently (Table 2), sometimes, though not always, in correlation with fleshy mesocarp. Of the basal genera, *Strempeliopsis*, *Haplophyton*, *Microplumeria*, *Aspidosperma*, and *Alstonia* have dry fruit. *Vallesia* has a fleshy drupe, and *Geissospermum* has a fleshy fruit, best described as a berry; and both *Neisosperma* and *Ochrosia* have a fleshy mesocarp. The dry, stringy layers in some genera, e.g., *Neisosperma*, are outgrowths of the endocarp; the mesocarp, however, is fleshy and rots away relatively quickly, leaving the thick endocarp outgrowths that form a thick, dry covering that serves for long-distance dispersal. The endocarp of *Aspidosperma* and *Vallesia* (Alstonieae sensu Endress & Bruyns, 2000) and *Neisosperma* and *Ochrosia* (Vinceae sensu Endress & Bruyns, 2000) is sclerified. Syncarpous *Lepiniopsis* and hemi-syncarpous *Rauvolfia serpentina* as well as the apocarpous Alyxieae and Plumerieae (sensu Endress

& Bruyns, 2000) have fleshy drupes with a sclerified endocarp; *Skytanthus* and *Cerberiopsis* have dry and fibrous mericarps, and *Alyxia* has fleshy drupe-like articles, which are detached, single-seeded portions of moniliform follicles (Middleton, 2000). Notably, taxa of the fleshy-fruited Carisseae have a non-sclerified endocarp. Sclerification of the endocarp is a specialization not well correlated with the bearing of fleshy drupes, as some sclerified taxa are certainly not drupaceous (e.g., *Aspidosperma*).

Seed presentation in the Apocynaceae s.l. is primitively naked, i.e., without a distinct aril and not embedded in a well-developed pulp (Table 2). The transition from naked seeds to seeds embedded in pulp appears to have occurred several times unambiguously within the Apocynaceae s.l., whereas the transformation from arillate to embedded-in-pulp has only occurred three times independently. The transition from naked to arillate is never unambiguous on the molecular-only tree (not shown), but very likely occurred in the ancestor of the Tabernaemontaneae clade. Independent origins of fruit pulp have occurred in *Geissospermum* (Alstonieae), *Couma* through *Saba* (Willughbeeae), *Molongum* (Tabernaemontaneae), *Melodinus* (Melodineae), Hunterieae, and the Carisseae. The arillate and pulp-embedded states are partly correlated with mesocarp fleshiness, conforming to the syndrome of features favoring animal-mediated seed dispersal.

The seed margin is primitively winged to naked in the Apocynaceae s.l. (Table 2). Wing homology is difficult to establish in phylogenetically basal and derived taxa. The question whether seed wings of the genus *Aspidosperma* (basal) are homologous to those found in the subfamilies Secamonoideae and Asclepiadoideae remains undecided, but these features were treated as homologous in this study. Independent origins of seed wings have occurred multiple times in the subfamilies Rauvolfioideae, Periplocoideae, and the Asclepiadoideae. Members of the genus *Aspidosperma* (Alstonieae) have the largest winged seed of the Apocynaceae s.l., and include both non-circumalate and circumalate seed. The wings of *Plumeria* seeds (Plumerieae) are non-circumalate, while those of *Strempeliopsis* (Alstonieae), *Tonduzia* [*Alstonia* (see Leeuwenberg, 1994) for a differing opinion], *Plectaneia*, and *Cerberiopsis*, although subject to different interpretation, were treated as having incompletely circumalate winged seeds. Of the members of the Periplocoideae, members of the genus *Mondia* have narrow seed wings (pers. obs.; see Swarupanandan et al., 1996, for a different opinion and for further discussion). As a rule, the seeds of members of the Asclepiadoideae have a very narrow flat margin that has sometimes been called winged, but homology to the large, well-defined wings in other parts of the family is uncertain. This character has been secondarily lost in a number of species in the following genera: *Matelea*, *Oxystelma*, *Gomphocarpus*, and *Hoya* (Stevens, pers. comm.).

The seed coma is primitively absent in the Apocynaceae s.l. (Table 2). The character state "ill-defined comas" refers to seed margins that surround the entire seed margin, are of uniform length, and are ciliate. Ill-defined comas have been defined as wings, but without thorough ontogenetic investigation these classifications are subject to interpretation; in this context, *Alstonia*, *Tonduzia*, and *Laxoplumeria* have ill-defined comas. Well-defined seed comas have localized areas that are highly pubescent; this character is present in the Apocynoideae, Periplocoideae, Secamonoideae, and the Asclepiadoideae. A well-defined seed coma appears to have been derived from an immediate ancestor with the syncarpous, fleshy-fruited members of the Carisseae (*Carissa* and *Acokanthera*), although this relationship is only moderately supported by a jackknife value of **81**/77% (Fig. 2, p. 534). The potential transition from a syncarpous fruit with seeds embedded in fleshy pulp to an apocarpous fruit with greatly increased numbers of small seeds with both wings and a well-defined coma was unexpected. The fleshy-fruited condition is most likely autapomorphous for the Carisseae. Given that most of the family has seeds with well-defined comas, the evolution of this character can be used to hypothesize the derivation of four of five extant subfamilies via radiative expansion.

The production of small seeds with a coma may have been the evolutionary step necessary for cladogenesis/speciation through increased dispersal and exposure to new habitats. As a likely result of coma-mediated seed dispersal, the Apocynaceae s.l. are found worldwide.

Geography in the Apocynaceae s.l. appears to be primitively Gondwanan (South America, Africa, and the Indian Ocean Basin) from non-additive optimization of area states (see Ross & Scotese, 1988, and Unternehr et al., 1988, for discussion on tectonic models; Ronquist, 1994; see Albert & Struwe, 1997, for an example of area optimization). This is useful information for estimating the age of origination for the genus *Aspidosperma* and its close neotropical associates, which must then have had a western Gondwanaland distribution.

Future research could be directed at integrated morphological and molecular-developmental studies of both pollination and seed dispersal traits to better understand the origins and mechanistic extents of both parallel and innovative reproductive evolution in this large angiosperm family.

Literature Cited

*An asterisk indicates a primary literature source for fruit and seed characters related to dispersal mode (see Table 2).

Albert, V. A. & L. Struwe. 1997. Phylogeny and classification of *Voyria* (saprophytic Gentianaceae). Brittonia 49: 466–479.

Allorge, L. 1980.* Considérations sur la systématique des Apocynacées. Pp. 112– 126 *in* L. Allorge, H. P. Husson & C. Sastre (editors), Morphologie et chimiotaxonomie des Apocynacées: Conclusions phylogénétiques et biogéographiques. Compt. Rend. Séances Soc. Biogéogr. 57.

———. 1985a.* Contribution à l'étude des graines des Apocynaceae–Tabernaemontanoideae: Origine de l'arille et ornementation du tegument séminal. Adansonia 7: 433–451.

———. 1985b.* Monographie des Apocynacées–Tabernaemontaoïdées Américaines. Morphologie, Systématique, Chimio-taxonomie. Mém. Mus. Natl. Hist. Nat., Sér. B, Bot. 30: 1–119.

Beentje, H. J. 1982.* A monograph on *Strophanthus* DC. (Apocynaceae). Meded. Landbouwhogeschool 83: 21–52.

Bergen, M. van & W. Snoeijer. 1996.* *Catharanthus*. The Madagascar periwinkle and related species. Wageningen Agric. Univ. Pap. 96(3): 1–120.

Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim van Dillen & J. van der Noordaa. 1990. Rapid and simple methods for purification of nucleic acids. J. Clin. Microbiol. 28: 495–503.

Brown, N. E. 1904.* Asclepiadeae. Pp. 231–503 *in* W. T. Thiselton-Dyer (editor), Flora of Tropical Africa 4(1). L. Reeve, London.

———. 1909.* Asclepiadeae. Pp. 518–1036 *in* W. T. Thiselton-Dyer (editor), Flora Capensis 4(1). L. Reeve, London.

Brown, R. 1810. On the Asclepiadeae, a natural order of plants separated from the Apocineae of Jussieu. [Preprint of Mem. Wern. Nat. Hist. Soc. 1: 12–78 (1811).]

Chávez de Michel, L. R. 1993.* Apocynaceae. Pp. 114–126 *in* T. J. Killeen, E. García E. & S. G. Beck (editors), Guia de Arboles de Bolivia. Editorial del Instituto de Ecología, La Paz, Bolivia.

Civeyrel, L. 1996. Phylogénie des Asclepiadaceae, approche Palynologique et Moléculaire. Ph.D. Dissertation, Montpellier.

———, A. le Thomas, K. Ferguson & M. W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *mat*K sequences. Molec. Phylogenet. Evol. 9: 517–527.

Conn, B. J. & E. A. Brown. 1996.* *Strychnos*. Pp. 64–66 *in* A. E. Orchard & A. Wilson (editors), Flora of Australia, Vol. 28. Gentianales. CSIRO Australia, Melbourne.

Degener, O. 1946.* Apocynaceae and Asclepiadaceae. Families 305 & 306 (no pages) *in* O. Degener (editor), Flora Hawaiiensis; The New Illustrated Flora of the Hawaiian Islands 1–4, Honolulu.

Demeter, K. 1922. Vergleichende Asclepiadeenstudien. Flora 115: 130–176.

Dilst, F. J. H. van. 1995.* *Baissea* A. DC. Series of revisions of Apocynaceae XXXIX. Bull. Jard. Bot. Belg. 64: 89–178.

Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Endress, M. E. 1995.* *Condylocarpon*. Pp. 492–493 *in* J. A. Steyermark, P. E. Berry & B. K. Holst (editors), Flora of the Venezuelan Guayana, Vol. 2. Timber Press, Portland, and Missouri Botanical Garden Press, St. Louis.

——— & P. V. Bruyns. 2000. A revised classification of Apocynaceae s.l. Bot. Rev. (Lancaster) 66: 1–56.

———, M. Hesse, S. Nilsson, A. Guggisberg & J. Zhu. 1990.* The systematic position of the Holarrheninae (Apocynaceae). Pl. Syst. Evol. 171: 157–185.

———, B. Sennblad, S. Nilsson, L. Civeyrel, M. W. Chase, S. Huysmans, E. Grafström & B. Bremer. 1996.* A phylogenetic analysis of the Apocynaceae s. str. and some related taxa in Gentianales: A multidisciplinary approach. Opera Bot. Belg. 7: 59–102.

Endress, P. K. 1994. Gentianales (Asteridae). Asclepiadaceae. Pp. 302–319 *in* Diversity and Evolutionary Biology of Tropical Flowers. Cambridge Univ. Press, Cambridge.

———, M. Jenny & M. E. Endress. 1983. Convergent elaboration of apocarpous gynoecia in higher advanced dicotyledons (Sapindales, Malvales, Gentiales). Nordic J. Bot. 3: 293–300.

Ezcurra, C. 1981.* Revisión de las Apocinaceas de la Argentina. Darwiniana 23: 367–474.

———, M. E. Endress & A. J. M. Leeuwenberg. 1992.* Apocynaceae. Pp. 1–120 *in* R. Spichiger & L. Ramella. (editors), Flora del Paraguay 17. Conservatoire et Jardin Botaniques de la Ville de Genève and Missouri Botanical Garden, St. Louis.

Fallen, M. E. 1983a.* A taxonomic revision of *Condylocarpon* (Apocynaceae). Ann. Missouri Bot. Gard. 70: 149–169.

———. 1983b. Morphological, Functional, and Evolutionary Aspects of the Flower in the Apocynaceae. Ph.D. Dissertation, Zurich.

———. 1985.* The gynoecial development and systematic position of *Allamanda* (Apocynaceae). Amer. J. Bot. 72: 572–579.

———. 1986. Floral structure in the Apocynaceae: Morphological, functional, and evolutionary aspects. Bot. Jahrb. Syst. 106: 245–286.

Farris, J. S. 1989. The retention index and homoplasy excess. Syst. Zool. 38: 406–407.

———, V. A. Albert, M. Källersjö, D. Lipscomb & A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12: 99–124.

Fitch, W. M. 1971. Toward defining the course of evolution: Minimum change for a specific tree topology. Syst. Zool. 20: 406–416.

Forster, P. I. & D. J. Liddle. 1996.* *Hoya, Dischidia*. Pp. 197–283 *in* A. E. Orchard & A. Wilson (editors), Flora of Australia, Vol. 28. Gentianales. CSIRO Australia, Melbourne.

Gentry, A. H. 1974.* Notes on Panamanian Apocynaceae. Ann. Missouri Bot. Gard. 61: 891–900.

———. 1983. *Alstonia* (Apocynaceae): Another paleotropical genus in Central America. Ann. Missouri Bot. Gard. 70: 206–207.

Hansen, B. F. 1995.* *Forsteronia*. Pp. 496–499 *in* J. A. Steyermark, P. E. Berry & B. K. Holst (editors), Flora of the Venezuelan Guayana, Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.

Hooker, J. D. 1882.* Apocynaceae. Pp. 621–672 *in* J. D. Hooker (editor), The Flora of British India 3(1). L. Reeve, London.

Hutchinson, J. & J. M. Dalziel. 1931.* Apocynaceae and Asclepiadaceae. Pp. 28–65 *in* J. Hutchinson & J. M. Dalziel (editors), Flora of West Tropical Africa 2. The Crown Agents for the Colonies, London.

Jarvie, J. K. & Ermayanti. 1997.* *Cerbera in* Tree Genera of Borneo—Descriptions and Illustrations [current web address: www.huh.harvard.edu/databases/legacy/navikey/phillipsia.html].

Jepson, W. L. 1923.* Apocynaceae and Asclepiadaceae. Pp. 768–775 *in* W. L. Jepson (editor), A Manual of the Flowering Plants of California. Univ. California Press, Berkeley.

Jhingan, A. K. 1992. A novel technology for DNA isolation. Meth. Molec. Biol. 3: 15–22.

Judd, W. S., R. W. Sanders & M. J. Donoghue. 1991. Angiosperm family pairs: Preliminary phylogenetic analyses. Harvard Pap. Bot. 1(5): 1–51.

Jussieu, A. L. de. 1789. Genera Plantarum. Herissant, Paris.

Kunze, H. 1993. Evolution of the translator in Periplocaceae and Asclepiadaceae. Pl. Syst. Evol. 185: 99–122.

———. 1990. Morphology and evolution of the corona in Asclepiadaceae and related families. Trop. Subtrop. Pflanzenwelt 76: 1–51.

———. 1996. Morphology of the stamen in the Asclepiadaceae and its systematic relevance. Bot. Jahrb. Syst. 118: 547–579.

———, U. Meve & S. Liede. 1994. *Cibirhiza albersiana*, a new species of Asclepiadaceae, and establishment of the tribe Fockeeae. Taxon 43: 367–376.

Kupicha, F. K. 1981.* Studies on African Apocynaceae: The genus *Acokanthera*. Kew Bull. 37: 40–67.

Leeuwenberg, A. J. M. 1983.* Some remarks on the taxonomy of the Plumerioideae (Apocynaceae). Bothalia 14: 799–801.

———. 1991.* A Revision of *Tabernaemontana*: The Old World Species. Royal Botanic Gardens Kew/Whitstable Litho, Whitstable.

———. 1994.* Taxa of the Apocynaceae above the genus level. Series of Apocynaceae XXXIII. Wageningen Agric. Univ. Pap. 94(3): 47–60.

———. 1995.* *Tabernaemontana*. Pp. 562–569 *in* J. A. Steyermark, P. E. Berry & B. K. Holst (editors), Flora of the Venezuelan Guayana, Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.

——— & P. W. Leenhouts. 1980. Taxonomy. Pp. 8–96 *in* A. J. M. Leeuwenberg (editor), A. Engler & K. Prantl's Die Natürlichen Pflanzenfamilien, Angiospermae: Ordnung Gentianales, Fam. Loganiacae, Vol. 28b. Duncker & Humblot, Berlin.

Liede, S. 1997.* Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae)—A synopsis. Taxon 46: 233–247.

Luckow, M. & A. Bruneau. 1997. Circularity and independence in phylogenetic tests of ecological hypothesis. Cladistics 13: 145–151.

Mabberley, D. J. 1997. The Plant Book: A Portable Dictionary of the Vascular Plants, 2nd ed. Cambridge Univ. Press, Cambridge.

MacFarlane, J. M. 1933.* The Apocynaceae and Asclepiadaceae. Pp. 1–181 *in* The Evolution and Distribution of Flowering Plants 1. Noel Printing, Philadelphia.

Maddison, W. P. & D. R. Maddison. 1992. MacClade: Analysis of phylogeny and character evolution. Ver 3.0. Computer program and documentation. Sinauer, Sunderland, Massachusetts.

Matthew, K. M. 1995.* Apocynaceae, Asclepiadaceae, Loganiaceae. Pp. 289–311 *in* K. M. Matthew (editor), An Excursion Flora of Central Tamilnadu, India. Balkema, Rotterdam.

Middleton, D. J. 1999.* Apocynaceae. *In* Flora of Thailand. Vol. 7(1): 1–153. The Forest Herbarium, Bangkok.

———. 2000. Revision of *Alyxia* (Apocynaceae). Part 1: Asia and Malesia. Blumea 45: 1–146.

Monachino, J. 1949.* A revision of the genus *Alstonia* (Apocynaceae). Pacific Sci. 3: 133–182.

Morillo, G. N. 1995.* *Catharanthus*, P. 491; *Galactophora*, Pp. 498–503; *Mandevilla*, Pp. 518–528; *Prestonia*, Pp. 548–550; *Rauvolfia*, Pp. 550–553; *Thevetia*, Pp. 570–571 *in* J. A. Steyermark, P. E. Berry & B. K. Holst (editors), Flora of the Venezuelan Guayana, Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.

Ngan, P. T. 1965.* A revision of the genus *Wrightia* (Apocynaceae). Ann. Missouri Bot. Gard. 52: 114–175.

Nilsson, S., M. E. Endress & E. Grafstrom. 1993. On the relationship of the Apocynaceae and Periplocaceae. Grana, Suppl. 2: 3–20.

Nowicke, J. W. 1970.* Apocynaceae. *In* R. E. Woodson & R. W. Schery (editors), Flora of Panama. Ann Missouri Bot. Gard. 57: 59–130.

Persoon, J. G. M., F. J. H. van Dilst, R. P. Kuijpers, A. J. M. Leeuwenberg & G. J. A. Vonk. 1992.* The African species of *Landolphia* P. Beauv. Series of revisions of Apocynaceae XXXIV. Wageningen Agric. Univ. Pap. 92(2): 1–232.

Pichon, M. 1947a. Classification des Apocynacées: IV. Genre *Alstonia* et genres voisins. Mém. Mus. Natl. Hist. Nat., sér. 2, 19: 294–301.

———. 1947b. Classification des Apocynacées: VIII. Les *Voacanga* d'Afrique. Mém. Mus. Natl. Hist. Nat., sér. 2, 19: 409–416.

———. 1948.* Classification des Apocynacées. I. Carissées et Ambelaniées. Mém. Mus. Natl. Hist. Nat., sér. 2, 21: 111–181.

———. 1949a.* Classification des Apocynacées: XXVI. Determination des echantillons fleuris de Plumerioidées. Mém. Mus. Natl. Hist. Nat., sér. 2, 21: 140–146.

———. 1949b.* Classification des Apocynacées: XXVII. Determination des graines de Plumerioidées et de Cerberoidées. Mém. Mus. Natl. Hist. Nat., sér. 2, 21: 266–269.

———. 1950a.* Classification des Apocynacées. XXV. Echitoïdées. Mém. Mus. Natl. Hist. Nat. Sér. B, 1: 1–174.

———. 1950b.* Classification des Apocynacées: XXV. Echitoïdées et Supplément aux Pluméroïées. Mém. Mus. Natl. Hist. Nat., Sér. B, 1: 1–174.

Ploeg, J. van der. 1983.* A Revision of *Isonema* R. Br. and *Pycnobotrya* Benth. (Apocynaceae). Wageningen Agric. Univ. Pap. 83(4): 1–20.

Potgieter, K. 1999. Phylogenetic Study of Apocynaceae Juss. and *Aspidosperma* Mart. & Zucc. Ph.D. Dissertation, University of Illinois, Urbana.

——— &. V. A. Albert. 1997. Preliminary phylogenetic study of the genus *Aspidosperma* (Apocynaceae) and closely related genera using ITS and *trn*L-F sequence data. Amer. J. Bot. 84 (6, Abstracts): 223.

——— & ———. 1998a. *Aspidosperma* evolution based on cladistic analyses of morphological and molecular (ITS, *trn*L-F) sequence data. Amer. J. Bot. 85 (6, Abstracts): 151.

——— & ———. 1998b. Non-floral morphological correlates of the Apocynaceae s.s.–Periplocaceae–Asclepiadaceae transition (i.e., dispersal traits) mapped onto a *trn*L-F derived consensus tree. Amer. J. Bot. 85 (6, Abstracts): 152.

Ronquist, F. 1994. Ancestral areas and parsimony. Syst. Biol. 43: 267–274.

Rosatti, T. J. 1989.* The genera of suborder Apocynineae (Apocynaceae and Asclepiadaceae) in the Southeastern United States. J. Arnold Arbor. 70: 307–401; 71: 443–514.

Ross, M. I. & C. R. Scotese. 1988. A hierarchical tectonic model of the Gulf of Mexico and Caribbean region. Tectonophysics 155: 139–168.

Rudijiman. 1986.* A revision of *Beaumontia* Wallich, *Kibatalia* G. Don and *Vallariopsis* Woodson (Apocynaceae). Wageningen Agric. Univ. Pap. 86(5): 3–44.

Safwat, F. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. Ann. Missouri Bot. Gard. 49: 95–129.

Sanderson, M. J. & J. J. Doyle. 1993. Phylogenetic relationships in North American *Astragalus* (Fabaceae) based on chloroplast DNA restriction site variation. Syst. Bot. 18: 395–408.

Savolainen, V., P. Cuénoud, R. Spichiger, M. D. P. Martinez, M. Crèvecoeur & J. F. Manen. 1995. The use of herbarium specimens in DNA phylogenetics: Evaluation and improvement. Pl. Syst. Evol. 197: 87–98.

Schlechter, F. R. R. 1905. Periplocaceae. Pp. 351–353 *in* K. Schumann & K. Lauterbach (editors), Nachträge zur Flora des Deutschen Schutzebiete in der Sudsee. Borntraeger, Leipzig.

Sennblad, B. 1997.* Is there a justification for differential *a priori* weighting in coding sequences?—A case study from *rbc*L and Apocynaceae. Ph.D. Dissertation, Acta Universitatis Upsaliensis, Uppsala.

——— & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.

——— & ———. 2000. Is there a justification for differential *a priori* weighting in coding sequence?—A case study from *rbc*L and Apocynaceae. Syst. Biol. 49: 43–55.

———, M. E. Endress & B. Bremer. 1998. Morphology and molecular data in phylogenetic fraternity—The tribe Wrightieae (Apocynaceae) revisited. Amer. J. Bot. 85: 1143–1158.

Sidiyasa, K. 1998. Taxonomy, phylogeny and wood anatomy of *Alstonia* (Apocynaceae). Blumea Suppl. 11: 1–230.

Small, J. K. 1903.* Apocynaceae and Asclepiadaceae. Pp. 933–956 *in* J. K. Small (editor), Flora of the Southeastern United States. [Published by the author] New York.

———. 1933.* Apocynaceae and Asclepiadaceae. Pp. 1057–1078 *in* J. K. Small (editor), Manual of the Southeastern Flora. New York.

Smith, A. C. 1988.* Apocynaceae and Asclepiadaceae. Pp. 43–123 *in* A. C. Smith (editor), Flora Viteinsis Nova. A New Flora of Fiji 4. Pacific Tropical Botanical Garden, Hawaii.

Souza, L. A. D. & I. S. Moscheta. 1992.* Morfo-anotomia do fruto e da plântula de *Aspidosperma polyneuron* M. Arg. (Apocynaceae). Revista Brasil. Biol. 52: 439–447.

Stapf, O. 1904.* Apocynaceae. Pp. 24–233 *in* W. T. Thiselton-Dyer (editor), Flora of Tropical Africa 4. L. Reeve, London.

Stevens, W. D. 1975. Notes on the genus *Matelea* (Apocynaceae, s.l.). Phytologia 32: 387–406.

———. 1988.* New names and combinations in Apocynaceae, Asclepiadoideae. Phytologia 64: 333–335.

Struwe, L. 1999. Morphological and Molecular Phylogenetic Studies in Neotropical Genetianaceae. Ph.D. Dissertation, Stockholm University, Sweden.

——— & V. A. Albert. 1997. Floristics, cladistics, and classification: Three case studies in Gentianales. Pp. 321–352 *in* J. Dransfield, M. J. E. Coode & D. A. Simpson (editors), Plant Diversity in Malesia III. Royal Botanical Gardens, Kew.

———, ——— & B. Bremer. 1994 [1995]. Cladistics and family level classification of the Gentianales. Cladistics 10: 175–206.

———, M. Thiv, J. W. Kadereit, A. S. R. Pepper, T. J. Motley, P. J. White, J. H. E. Rova, K. Potgieter & V. A. Albert. 1998. *Saccifolium* (Saccifoliaceae), an endemic of Sierra de la Neblina on the Brazilian-Venezuelan frontier, is related to a temperate-alpine lineage of Gentianaceae. Harvard Pap. Bot. 3(2): 199–214.

Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996.* The subfamilial and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.

Swofford, D. L. 1998. PAUP-Phylogenetic Analysis Using Parsimony (and Other Methods), version 4.0.0d64. Computer program distributed by Swofford & Sinauer, Sunderland, Massachusetts.

Taberlet, P., L. Gielly, G. Patou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Molec. Biol. 17: 1105–1109.

Thorne, R. F. 1992. Classification and geography of the flowering plants. Bot. Rev. 58: 225–348.

Unternehr, P., D. Curie, J. L. Olivet, J. Goslin & P. Beuzart. 1988. South Atlantic fits and intraplate boundaries in Africa and South America. Tectonophysics 155: 169–179.

Vonk, G. J. A. & A. J. M. Leeuwenberg. 1989.* A taxonomic revision of the genus *Tabernanthe* and a study of wood anatomy of *T. iboga*. Wageningen Agric. Univ. Pap. 89(4): 3–18.

Williams, J. B. 1996.* *Manderilla* and *Parsonsia*. Pp. 104–196 *in* A. E. Orchard & A. Wilson (editors), Flora of Australia, Vol. 28. Gentianales. CSIRO Australia, Melbourne.

Woodson, R. E., Jr. 1935.* Studies in the Apocynaceae IV. The American genera of Echitoideae V. *Forsteronia*, etc. Ann. Missouri Bot. Gard. 22: 153–306.

———. 1936.* Studies in the Apocynaceae IV. The American genera of Echitoideae. Ann. Missouri Bot. Gard. 23: 169–438.

———. 1938.* Apocynaceae. N. Amer. Flora 29: 103–192.

Zarucchi, J. L. 1987.* Revision of the Tribe Ambelanieae (Apocynaceae–Plumerioideae). Wageningen Agric. Univ. Pap. 87(1): 22–89.

———. 1995.* *Couma*, Pp. 494–495; *Himatanthus*, Pp. 505–508; *Lacmellea*, Pp. 509–511; *Macoubea*, Pp. 511–512; *Microplumeria*, Pp. 531–532; *Molongum*, Pp. 532–533; *Pacouria*, Pp. 542–543; *Plumeria*, Pp. 546–547 *in* J. A. Steyermark, P. E. Berry & B. K. Holst (editors), Flora of the Venezuelan Guayana Vol. 2. Timber Press, Portland, & Missouri Botanical Garden Press, St. Louis.

Appendix 1. List of accessions sampled for DNA used for the Apocynaceae s.l. plastic *trn*L intron and *trn*L-F spacer study. Subfamilial and tribal designations are provided in the first two columns. Sample identification, place of collection (where known), and voucher information are given in the next three columns. GenBank accession numbers for deposited *trn*L intron and *trn*L-F spacer sequences are given in the last two columns, respectively. Specimen voucher information includes collector and collection number, location of voucher and/or living material. Herbarium acronyms follow Index Herbariorum: AAU, BR, C, CONN, DAV, FTG, GA, ILL, MO, NOU, NY, WAG, Z (* = permanent anatomical slides of flowers: M. Endress).

Outgroups		Loganiaceae	Country	Voucher	GenBank Sequence	
		Bonyunia superba M. R. Schomb. ex Progel	Venezuela, Bolivar	*Berry & Brako 5525* (NY)	AF102386	AF214146
		Strychnos tomentosa Benth.	French Guiana, Région de Saül	*Mori 24166* (NY)	AF102484	AF214147
					GenBank Sequence	
Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	*trn*L	*trn*L-F
Apocynoideae	Apocyneae	*Apocynum androsaemifolium* L.	U.S.A.	*Potgieter & Cahilly 238* (NY)	AF214308	AF214154
		Apocynum cannabinum L.	U.S.A.	*Potgieter & Cahilly 239* (NY)	AF102380	AF214155
		Baissea leonensis Benth.	Cameroun, Etonde-Fang	*DeKruif 1067* (WAG)	AF214320	AF214166
				81-0058 (BR)		
		Beaumontia grandiflora Wall.		890007 (CONN)	AF214321	AF214167
		Chonemorpha fragrans (Moon) Alston		*Forster 20091* (Z*)	AF214334	AF214180
				941310 (FTG)	AF214335	AF214181
				07-3805, *Billiet 3530* (BR)	AF214336	AF214182
		Forsteronia corymbosa (Jacq.) G. Mey.	Puerto Rico	*Struwe 1093* (NY)	AF214355	AF214201
		Trachelospermum jasminoides Lem.		851419 (CONN)	AF214439	AF214285
		Urceola rosea (Hook. & Arn.) D. J. Middleton		*Forster 20090* (Z*)	AF214445	AF214291
	Echiteae	*Cycladenia humilis* Benth.	U.S.A., California	*Smith & Rultan 520* (Z)	AF214341	AF214187
		Echites umbellata Jacq.		*Correll 45549* (NY)	AF214350	AF214196
		Parsonsia eucalyptophylla F. Muell.	Australia, Queensland	*Forster 20118* (Z*)	AF214395	AF214241
		Peltastes peltatus (Vell.) Woodson	Paraguay, Alto Parana	*Billiet & Jadin 3477* (Meise)	AF214396	AF214242
				84-2849, *Billiet 3526* (BR)		
		Pentalinon luteum (L.) B. F. Hansen & R. P. Wunderlin	Australia, Brisbane	Forster 20089 (Z*)	AF214397	AF214243
			Puerto Rico	*Struwe 1114* (NY)	AF214446	AF214292
		Prestonia mollis HBK		*Zona 833* (FTG), 94984 (FTG)	AF214410	AF214256
		Prestonia quinquangularis (Jacq.) Spreng.		51-0287, *Billiet 205* (BR)	AF214411	AF214257
	Malouetieae	*Funtumia elastica* (Preuss) Stapf	Congo, Eala	*Couteaux s.n.* 37-3045, *Billiet 3126* (BR)	AF214357	AF214203
		Holarrhena pubescens H. P. Wall. & G. Don		*Tawakali 1478* (MO)	AF214366	AF214212
		Mascarenhasia arborescens A. DC.		*Meagher 998* (FTG) 4376 (FTG)	AF214378	AF214224

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					*trn*L	*trn*L-F
	Mesechiteae	*Galactophora crassifolia* (Muell.-Arg.) Woodson		*Romero 1629* (NY)	AF214358	AF214204
		Mandevilla sp.	Brazil, Jacobina	*Amorium 1806* (NY)	AF214376	AF214222
	Wrightieae	*Isonema smeathmanni* Roem. & Schult.	West Africa, Abouabou	*Oldeman s.n.* (WAG) 1964-0527, *Billiet 119* (BR)	AF214370	AF214216
		Nerium oleander L.		*Potgieter 255* (NY)	AF214386	AF214232
		Strophanthus gratus Franch.	Tropical Africa	850451 (CONN)	AF214430	AF214276
		Wrightia tomentosa Roem. & Schult.		*Gillis 10903* (FTG), 57157 (FTG)	AF214453	AF214299
Asclepiadoideae	Asclepiadeae	*Asclepias curassavica* L.	U.S.A.	*Potgieter & Cahilly 243* (NY)	AF102381	AF214156
		Asclepias syriaca L.	U.S.A.	*Potgieter & Cahilly 241* (NY)	AF214311	AF214157
		Asclepias tuberosa L.	U.S.A.	*Potgieter & Cahilly 242* (NY)	AF214312	AF214158
		Calotropis procera (Aiton) W. T. Aiton	Puerto Rico	*Struwe 1095* (NY)	AF214324	AF214170
		Cynanchum sp.		72-2017 *Billiet 2320* (BR)	AF214342	AF214188
		Ditassa sp.	Venzuela, Amazonas	*Maguire 28465* (NY)	AF102412	AF214190
		Gomphocarpus physocarpus E. Mey.		P1965-5572 (C)	AF214360	AF214206
		Gomphocarpus sp.	U.S.A., Hawaii	*Struwe & Albert 1194* (NY)	AF214361	AF214207
		Gonolobus patens Decne.	Ghana, Western	*Schmidt 2031* (MO)	AF214362	AF214208
		Matelea sp.	Argentina, Jujuy	*Taylor 11166* (MO)	AF214379	AF214225
		Oxystelma bornouense R. Br.	Ghana, Accra	*Schmidt 1724* (MO)	AF214392	AF214238
		Pachycarpus sp.		*Potgieter 257* (NY)	AF214393	AF214239
		Philibertia gilliesii Hook. & Arn.		s1558-2389 (C)	AF214401	AF214247
		Sarcostemma mulanjense S. Liede & U. Meve		32AS13 (GA)	AF214418	AF214264
		Sarcostemma stolonifera B. R. Adams & R. W. K. Holland		32AS14 (GA)	AF214419	AF214265
		Tweedia coerulea D. Don ex Sweet		34512 3451-2 (C)	AF214443	AF214289
		Tylophora sylvatica Decne.	Uganda	*Stevens 3752* (MO)	AF214444	AF214290
		Vincetoxicum hirundinaria Medic.	Switzerland	*Endress s.n.* (Z*)	AF214450	AF214296
		Vincetoxicum nigrum (L.) Moench		*Potgieter 256* (NY)	AF214451	AF214297
	Ceropegieae	*Caralluma crenulata* Wall.		p1984-5290-3523 (C)	AF214325	AF214171
		Caralluma gerstneri Letty		1992-0097-80 (BR)	AF214391	AF214237
		Ceropegia sandersoni Decne. ex Hook.		*Potgieter 248* (NY)	AF214333	AF214179
		Duvalia elegans (Masson) Haw.		1979-0578, EtOH (BR)	AF214347	AF214193
		Duvaliandra dioscoridis (Lavranos) M. G. Gilbert		1993-0034-26, EtOH (BR)	AF214346	AF214192
		Echidnopsis cereiformis Hook.		*Potgieter 253* (NY)	AF214348	AF214194
		Echidnopsis dammanniana Sprenger	Kenya	*Bamps s.n.*, 78-0098, EtOH (BR)	AF214349	AF214195

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					*trn*L	*trn*L-F
		Frerea indica Dalzell		p1995-5118 3523B (C)	AF214356	AF214202
		Huernia keniensis R. E. Fries	Kenya	852628 (CONN)	AF214368	AF214214
		Ophionella arcuata (N. E. Br.) Bruyns var. *mirkinii* (Pillans) Bruyns	South Africa	1987-0433, EtOH (BR)	AF214388	AF214234
		Orbea variegata (L.) Haw.	South Africa	19852630 (CONN)	AF214390	AF214236
		Orbeanthus hardyi (R. A. Dyer) L. C. Leach		p1980-5518-3524N-1 (C)	AF214389	AF214235
		Piaranthus geminatus (Masson) N. E. Br.		*Camboulive s.n.* 1979-0712 (BR)	AF214402	AF214248
				p-1994-5328 3526 (C)	AF214403	AF214249
		Stapelia leendertziae N. E. Br.		*Gilbert s.n.* (NY)	AF214424	AF214270
		Stapelia peglere N. E. Br.		32AS16 (GA)	AF212483	AF214271
		Stapelia revoluta Masson		*Potgieter 246* (NY)	AF214426	AF214272
		Stapelia schinzii Berger & Schltr.		p1980-5515-3524 (C)	AF214427	AF214273
		Tavaresia grandiflora (K. Schum.) Berger		p1990-5312 (C)	AF214433	AF214279
		Trichocaulon triebneri Nel		890032 (CONN)	AF214440	AF214286
		Tridentea longipes (C. A. Lückh.) Leach	South Africa, Richtersveld	1992-0113-96 (BR)	AF214441	AF214287
		Tromotriche revoluta (L.) Haw.		p-1980-5514-3524L-1 (C)	AF214442	AF214288
	Marsdenieae	*Dischidia bengalensis* Colebr.		920392 (CONN)	AF214343	AF214189
		Dregea sinensis Hemsl.		p-1972-5680 (C)	AF214345	AF214191
		Fockea cylindrica R. A. Dyer		p1994-5151 (C)	AF214352	AF214198
		Fockea edulis K. Schum.		*Potgieter 249* (NY)	AF214353	AF214199
		Fockea sinuata (E. Mey.) Druce		*Bourdoux s.n.* 1980-0551, EtOH (BR)	AF214354	AF214200
		Hoya australis R. Br. ex Traill		*Potgieter 247* (NY)	AF214367	AF214213
		Marsdenia amorimii G. Morillo	Brazil, Bahia	*Amorim 911* (NY)	AF214377	AF214223
		Micholitzia obcordata N. E. Br.	Thailand	*Seidenfaden 1972-5599* (C)	AF214381	AF214227
		Telosma cordata (Burm. f.) Merr.		*Gilding s.n.* (NY)	AF102493	AF214280
Periplocoideae		*Cryptolepis oblongifolia* (Meisn.) Schltr.	Africa	*Albers 543* (MO)	AF214422	AF214268
		Cryptostegia grandiflora R. Br.	U.S.A., Hawaii	*Struwe & Albert 1217* (NY)	AF214340	AF214186
		Ischnolepis natalensis (Schltr.) Venter		77BI306 (AAU, Z*)	AF214400	AF214246
		Mondia whiteii Skeels		*Potgeiter 258* (NY) 2717/94A (NY)	AF214384	AF214230

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence	
					*trn*L	*trn*L-F
		Periploca graeca L.		*Endress s.n.* (Z*)	AF102468	AF214244
		Stomatostemma monteiroae N. E. Br.		*Potgieter 257* (NY), 1320/93A (NY)	AF214428	AF214274
Rauvolfioideae	Alstonieae	*Alstonia boonei* De Wild.	Ivory Coast	*Leeuwenberg 10724* (WAG)	AF102374	AF214151
		Aspidosperma australe Müll. Arg.	Bolivia	*Jardim s.n.* (MO)	AF214313	AF214159
		Aspidosperma cuspa (Kunth) Blake	Colombia, Bolivar	*Gentry 47406* (NY)	AF214315	AF214161
		Aspidosperma desmanthum Benth. ex Müll. Arg.	Belize, Orange Walk	*Whitefoord 8023* (MO)	AF214314	AF214160
		Aspidosperma megalocarpon Müll. Arg.	Mexico	*Breedlove 50932* (MO)	AF102382	AF214162
		Aspidosperma myristicifolium (Markgraf) Woodson	Costa Rica	*Aguilar & Potgieter 3555* (MO)	AF214317	AF214163
		Aspidosperma pyrifolium Mart.	Bolivia	*de Queiroz 4136* (NY)	AF214318	AF214164
		Aspidosperma quebracho-blanco Schltdl.	Bolivia, Santa Cruz	*Bettella 165* (MO)	AF214319	AF214165
		Geissospermum laeve Miers	Brazil, Linhares	*Farias 23* (CVRD, Z*)	AF214359	AF214205
		Haplophyton crooksii (L. D. Benson) L. D. Benson	U.S.A., Arizona	*Gilbertson s.n.* (Z*)	AF214363	AF214209
		Laxoplumeria baehniana Monach.	French Guiana, Région de Saül	*Mori 20933* (NY)	AF214373	AF214219
		Microplumeria anomala (Müll.-Arg.) Markgraf	Brazil, Amazonas	*Zarucchi 3116* (NY)	AF214382	AF214228
		Strempeliopsis strempelioides Benth.	Cuba, Sanctus Espiritus	*Acevedo-Rodriguez 6444* (NY)	AF214429	AF214275
		Tonduzia longifolia (DC.) Markgraf	Costa Rica, San Jose	*Morales 2776* (NY)	AF214437	AF214283
		Tonduzia stenophylla (Donn. Sm.) Pittier	Guatemala, Izabal	*Marshall 267* (NY)	AF214438	AF214284
		Vallesia antillana Woodson		*Zona 710* (FTG)	AF214447	AF214293
	Alyxieae	*Alyxia buxifolia* R. Br.		s1981-0824-3393-2 (C)	AF214306	AF214152
		Condylocarpon amazonicum (Markgraf) Ducke	French Guiana, Région de Saül	*Mori 20860* (NY)	AF214337	AF214183
		Condylocarpon sp.	Brazil, Ilheus	*Thomas 9956* (NY)	AF214338	AF214184
		Lepiniopsis ternatensis Valeton	U.S.A., Caroline Island	*Kanehira 566* (NY)	AF214374	AF214220
		Plectaneia stenophylla Jum.	Madagascar	*H. Petignat* (Z*)	AF214405	AF214251
					AF214406	AF214252
		Pteralyxia kauaiensis Caum	U.S.A., Hawaii	*Lorence 7768* (NY, Z*)	AF214412	AF214258

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence *trn*L	GenBank Sequence *trn*L-F
	Carisseae	*Acokanthera oppositifolia* (Lam.) Codd	South Africa, Eastern Province	*Bayliss 544* (NY)	AF214302	AF214148
		Acokanthera rotundata (Codd) Kupicha	Spain, Canary Islands	*Wiese s.n.* (WAG) 80-0032, *Billiet 1875* (BR)	AF214303	AF214149
		Carissa bispinosa (L.) Desf. ex Brenan		1953-3766, *Billiet 3529* (BR)	AF102391	AF214172
		Carissa carandas L.		77780 (FTG)	AF214327	AF214173
	Hunterieae	*Hunteria umbellata* Hallier f.		*Endress 97-16* (Z)	AF214369	AF214215
		Picralima nitida (Stapf) T. Durand & H. Durand	D. R. Congo	*Kiss s.n.* 86-0334, *Billiet 3440* (BR)	AF214404	AF214250
		Pleiocarpa mutica Benth.		19610253, *Billiet 1766* (Z*, BR)	AF214407	AF214253
	Melodineae	*Melodinus monogynus* Roxb.		1939-1959, *Billiet 3359* (Z*, BR)	AF214380	AF214226
	Plumerieae	*Allamanda* sp.		*Wurdack s.n.* (NY-1956)	AF214304	AF214150
		Cerbera manghas L.	U.S.A., Hawaii	*Endress (s.n.)* (Z*)	AF214330	AF214176
		Cerbera odollam Gaertn.	U.S.A., Hawaii	*Struwe & Albert 1216* (NY)	AF214331	AF214177
		Cerberiopsis candelabra Vieill.	New Caledonia, Noumea	*Tanguey 3288* (NOU)	AF102395	AF214178
		Himatanthus sp.	Brazil, Acre	*Silveira 1336* (NY)	AF214364	AF214210
		Himatanthus tarapotensis (K. Schum. ex Markgr.) Plumel	Brazil, Acre	*Cid 10241* (NY)	AF214365	AF214211
		Plumeria alba Kunth	Puerto Rico	*Struwe 1096* (NY)	AF214408	AF214254
		Plumeria obtusa L.	Guatemala	*Specht s.n.* (NY)	AF214409	AF214255
		Skytanthus acutus Meyen	Chile	*Grosjean s.n.* (Z*)	AF214423	AF214269
		Thevetia ahouai (L.) A. DC.		*Zona 618* (FTG, Z*), 95-529 (FTG)	AF214435	AF214281
		Thevetia peruviana (Pers.) K. Schum.		*Watson 1473* (FTG), 81393 (FTG) *Struwe & Albert 1213* (NY)	AF214328	AF214174
			U.S.A., Hawaii		AF214436	AF214282
	Tabernaemontaneae	*Bonafousia siphilitica* (L. f.) L. Allorge	French Guiana, Région de Saül	*Phillippe 26927* (NY)	AF214322	AF214168
		Callichilia barteri (Hook. f.) Stapf		*Van Stellen 116* (WAG) 1982-0466, *Billiet 3527* (BR)	AF214323	AF214169
		Macoubea guianensis Aubl.	Brazil, Linhares	*Folli 5* (CVRD, Z)	AF214375	AF214221
		Molongum laxum (Benth.) Pichon	Brazil, Amazonas	*Rodrigues 10741* (NY)	AF214383	AF214229
		Tabernaemontana citrifolia L.	Puerto Rico, Rio Grande	*Taylor 11858* (MO)	AF214431	AF214277
		Tabernaemontana floribunda Blume	U.S.A., Florida	*Fantz 3161* (FTG) 591079 (FTG)	AF214351	AF214197

Appendix 1. Continued.

Subfamily	Tribe	Apocynaceae s.l.	Country	Voucher	GenBank Sequence *trn*L	GenBank Sequence *trn*L-F
		Tabernaemontana Plum.	Brazil, Acre	*Daly 603* (NY)	AF214399	AF214245
		Tabernanthe iboga Baill.		1981-0308, *Billiet 3557* (BR, Z*)	AF214432	AF214278
		Voacanga africana Stapf	Ivory Coast, Sassandra	*Leeuwenberg 12054* (WAG) 80-0066, *Billiet 3528* (BR)	AF214452	AF214298
	Vinceae	*Amsonia orientalis* Decne.		3403 (C)	AF214416	AF214262
		Amsonia tabernaemontana Walter	U.S.A.	*Potgieter & Cahilly 237* (NY)	AF214307	AF214153
		Catharanthus roseus G. Don		*Potgieter 245* (NY)	AF102392	AF214175
		Neisosperma nakiana (Koidzumi) F. R. Fosberg & M.-H. Sachet		*Endress 884* (Z*)	AF214385	AF214231
		Ochrosia elliptica Labill.		*Gillis 7573* (FTG, Z*) X-2-101 (FTG)	AF214387	AF214233
		Rauvolfia balansae (Baill.) Boit.	New Caledonia	*Lowry 4760* (MO)	AF214413	AF214259
		Rauvolfia serpentina Benth. ex Kurz		*Potgieter 252* (NY)	AF214414	AF214260
					AF214415	AF214261
		Vinca difformis Pourr.		(DAV)	AF214448	AF214294
		Vinca minor L.		*Potgieter 256* (NY)	AF214449	AF214295
	Willughbeeae	*Couma macrocarpa* Barb. Rodr.	Colombia	*Cárdenas 7379* (Z)	AF214339	AF214185
		Lacmellea aculeata (Ducke) Monachino	Brazil, Bahia	*Amorim 1229* (NY)	AF214371	AF214217
		Landolphia incerta (K. Schum.) Persoon	Eastern Ghana	*Schmidt 1696* (MO)	AF214372	AF214218
		Pacouria guianensis Aubl.	French Guiana, Région de Saül	*Allorge 337* (NY)	AF214394	AF214240
		Saba comorensis (Bojer) Pichon		00-5412, *Billiet 3442* (BR)	AF214417	AF214263
Secamonoideae		*Secamone glaberrima* K. Schum.	Madagascar, Toocmasina	*Stevens 26007* (MO)	AF214420	AF214266
		Secamone oleaefolia Decne.	Madagascar, Antananarivo	*Stevens 26005* (MO)	AF214421	AF214267

DIVERSITY AND RELATIONSHIPS WITHIN THE PERIPLOCOIDEAE (APOCYNACEAE)[1]

H. Johan T. Venter[2] *and*
Rudolf L. Verhoeven[2]

ABSTRACT

Morphological diversity and phylogenetic relationships in the Periplocoideae are analyzed. Most of the species of this subfamily inhabit tropical or subtropical forests and savannas as woody climbers, woody shrubs, epiphytes, or herbaceous geophytes. The flower is complex and has evolved into many forms, always incorporating a corolline corona, interstaminal corona-like nectaries, and stamens fused with the style-head into a gynostegium. Pollen is borne in tetrads, less often as pollinia, which are shed onto spathulate translators that are secreted in grooves around the periphery of the style-head. Phylogenetic relationships among the Periplocoideae genera are analyzed cladistically using morphological characters, mainly of the flower. This analysis reveals one larger clade and two smaller clades. Eleven new generic synonyms, six new specific synonyms, and fifteen new species combinations are required within the subfamily. A key to the genera of the Periplocoideae is included.

Key words: Apocynaceae, generic key, morphological diversity, nomenclatural corrections, Periplocoideae, phylogeny.

The Periplocoideae (Apocynaceae) are restricted to the Old World, in Africa, Madagascar, Europe, Asia, and Australia, mainly in tropical and subtropical regions, with the majority of the species found in tropical evergreen rainforest, tropical seasonal (monsoon) forest, and tropical woodlands (savannas) (Venter et al., 1990a; Venter, 1997; Venter & Verhoeven, 1996a). A small number of taxa, e.g., *Raphionacme galpinii* Schltr. and *R. hirsuta* (E. Mey.) R. A. Dyer, inhabit the grasslands, and a few taxa are found in desert and semi-desert habitats (e.g., the three species of *Ectadium* E. Mey., *Periploca aphylla* Decne., *P. visciformis* (Vatke) K. Schum., *Raphionacme haenelii* Venter & R. L. Verh., and *R. namibiana* Venter & R. L. Verh. (Venter, 1997; Venter & Verhoeven, 1986b, 1996b; Venter et al., 1990b)). Interestingly, no Periplocoideae inhabit the South African Cape Floral Kingdom with its winter rainfall. However, a few species, e.g., *Periploca angustifolia* Labill. and *P. gracilis* Boiss., occur in the Mediterranean macchia of North Africa and Europe (Venter, 1997).

At present the Periplocoideae comprise 31 genera and 181 species. Most of the genera comprise only a few species. The largest genera are *Raphionacme* Harv. (37 species), *Cryptolepis* R. Br. (27 species), *Pentopetia* Decne. (21 species) (Klackenberg, 1999), and *Periploca* L. (13 species) (Venter, 1997). The difference between these numbers and the 44 genera and ca. 190 species given by Venter and Verhoeven (1997) in their classification of the subfamily is because a number of little known genera, most of them monotypic and from Asia, have been put into synonymy since then. Two genera have been placed in synonymy by Klackenberg (1997, 1998, 1999) and another 11 genera are synonymized here (one genus previously incorrectly synonymized and corrected here) (Appendices 2 & 3). During the same time 14 new species have been added (Klackenberg, 1997, 1998, 1999; Venter & Verhoeven, 1999, 2000), a number of existing ones have been placed in synonymy (Klackenberg 1997, 1998, 1999), and another 6 are synonymized in the present article (Appendix 3). Fifteen new combinations are validated herein (Appendix 3).

The majority of periplocoid taxa are woody climbers of moist or drier forests or savanna, such as *Buckollia* Venter & R. L. Verh. in Africa (Venter & Verhoeven, 1994b), *Cryptolepis* (in part) in Africa and Asia, and *Gymnanthera* R. Br. in Asia and Australia (Forster, 1991). Some of the climbers, e.g., *Mondia* Skeels and *Tacazzea* Decne., both

[1] The University of the Free State, Bloemfontein, and the National Research Foundation, Pretoria, are thanked for their financial support toward the revision of the Periplocoideae. We also extend thanks to the herbaria, mentioned under Materials and Methods, for the use of their specimens and libraries, to Rouvay Roodt, who carried out the cladistical analysis, and to Johan Spies for his comments on the resultant cladogram. We are deeply grateful to Mary Endress for her valuable comments and assistance.

[2] Department of Botany and Genetics, University of the Free State, P.O. Box 339, Bloemfontein, South Africa. venterhj@sci.uovs.ac.za.

widely spread over Africa, are large lianas extending into the forest canopies of tropical rain and swamp forests. *Finlaysonia obovata* Wall. is restricted to mangrove forest in Asia and Australia. Some genera, such as *Cryptolepis* (in part) and *Periploca* (in part), are erect or straggling shrubs found in semi-arid habitats in northern Africa and western Asia. The erect shrub *Ectadium* occurs in true desert in southwestern Africa. *Baroniella* Costantin & Gallaud (found in Madagascar and the Mascarenes), *Camptocarpus* Decne. (endemic to Madagascar) (Klackenberg, 1997, 1998), and *Raphionacme* Harv. (from Africa and Arabia) are suffrutescent taxa, the first two genera small forest climbers and the latter genus mostly herbaceous geophytes found in open seasonal swamps, grasslands, savannas, semi-desert, or true desert. *Epistemma*, which has three species, and *Sarcorrhiza*, which is monotypic, are epiphytes in tropical forests of Africa.

Periplocoideae are never dominant in the plant communities where they occur, although lianas like *Mondia* and *Tacazzea* may be conspicuous because of their size. The taxa are often widely distributed, but large numbers of individual plants seldom occur. The exception is *Cryptostegia grandiflora* Roxb. ex R. Br. of Madagascar, which has become common in disturbed situations in Madagascar. In Queensland, Australia, it has become a major noxious weed of grazing lands (Marohasy & Forster, 1991).

The aim of the present article is to describe the unique characteristics of the Periplocoideae, to update their nomenclature, to provide a key to the 31 genera acknowledged, and to analyze the phylogenetic relationships among these genera cladistically.

Materials and Methods

Taxon Sampling

Roots, stems, leaves, inflorescences, flowers, fruit, and seed of the Periplocoideae, representing all 31 genera currently recognized in the subfamily, were investigated. As very little fresh or spirit material was available, dried flowers from herbarium specimens were rehydrated for analysis by soaking the flowers in a hot, but not boiling, weak solution of dishwashing liquid for 5 to 15 minutes depending on the thickness and compression of the flower parts. Material from the following herbaria was studied: ABD, B, BLFU, BM, BOL, BR, BRI, COI, E, FT, G, GRA, K, L, LAE, LINN, LISC, M, MO, NBG, NH, NU, OXF, P, PRE, QRS, S, SRGH, UPS, W, WAG, WIND, and Z. Examination was carried out with an Olympus SZ-PT Stereo Microscope, a Zeiss Light Microscope, and a Jeol Winsem 6400 Electron Microscope.

The outgroup taxa for this study, *Apocynum* L. and *Parsonsia* R. Br., were chosen as two of the putatively closest relatives of the Periplocoideae, among the Apocynoideae, Apocynaceae (Nilsson et al., 1993; Sennblad & Bremer, 1996; Sennblad et al., 1998; Sennblad & Bremer, 2000; Potgieter & Albert, 2001 this volume).

Morphology

Thirty-nine characters were coded and scored for the 33 genera. The characters are based on pooled results of all species in the genus, with the most common character states being coded for. We feel this approach is no more susceptible to artifacts than is the exemplar method, especially given the fact that more than 50% of all genera contain only 1 or 2 species, and only four (*Cryptolepis*, *Pentopetia*, *Periploca*, and *Raphionacme*) contain more than 10 species, so that most genera are reasonably homogeneous. Where ambiguities in coding arose, these are discussed in the section on Character States.

Analysis

The 31 taxa of the ingroup plus the two outgroup taxa were included in the matrix, which was subsequently analyzed using parsimony methods. A cladistic analysis was carried out using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b4a (Swofford, 1998). Searches for islands of most parsimonious trees were conducted as described by Katz-Downie et al. (1999). The lengths of the shortest trees were determined by generating 100 heuristic searches using random addition starting trees, with tree bisection-reconnection (TBR) branch swapping and MULPARS selected, but saving no more than one of the shortest trees from each search. These trees were then used as starting trees for TBR branch swapping (with MULPARS and steepest descent selected). The strict consensus from the obtained trees was then saved as topological constraint (Catalan et al., 1997). One hundred (100) random order entry replicate searches were then initiated again, saving no more than one tree from each search. In this analysis, however, only trees that did not fit the topological constraint were saved. No additional minimum length trees were found, which indicates that the strict consensus tree adequately summarizes available data. Bootstrap values were calculated from 100 replicate analyses using a heuristic search strategy, simple taxon ad-

dition sequence, and TBR branch swapping with all characters equally weighted. Owing to the relatively large size of the data set and the many resultant minimal length trees, a maximum tree limit of 100 trees/replicate was set. One tree was held during each step with the steepest descent and MULPARS options in effect. Decay values were calculated by increasing the tree length accordingly, following the methods of Bremer (1988) and Donoghue et al. (1992). Heuristic searches were conducted using 100 replicate analyses, TBR branch swapping, and MULPARS selected. Decay values could, however, only be calculated up to one step due to insufficient memory.

Results

Morphology

Roots. The root is the least known organ of the Periplocoideae. However, it is known that roots are unmodified in some taxa (e.g., *Ectadium*, *Cryptolepis*, and *Periploca*), while in others, such as *Mondia*, *Ischnolepis* Jum. & H. Perrier, *Raphionacme*, *Sacleuxia* Baill., and *Schlechterella* K. Schum., tuberous roots are found. These roots are usually more or less cylindrical, but in *Raphionacme* the single tuber is, with a few exceptions, uniquely turnip-shaped. The second author (Verhoeven, unpublished results) investigated the tuber of *Raphionacme procumbens* Schltr. anatomically and found that the crown of the tuber, from which the aerial stems arise, has typical stem anatomy, but lower down the tuber exhibits root anatomy.

The tubers of some taxa are used as medicine, such as those of *Mondia whitei* (Hook. f.) Skeels for abdominal ailments, poor appetite, schistosomiasis, as an aphrodisiac, etc. (Hutchings et al., 1996), and the "strongly vanilla scented" roots of *Tacazzea conferta* N. E. Br. as a lactogogue (Bullock, 1955). Chemical tests on the tuber of *Raphionacme galpinii* Schltr. look promising for anti-viral and anti-cancerous properties (pers. comm., J. Harington). Two alkaloids, cryptolepine and isocryptolepine, extracted from the roots of *Cryptolepis sanguinolenta* (Lindl.) Schltr. show inhibitory activity against the human malaria parasite, *Plasmodium falciparum* (Grellier et al., 1996).

Stems. The climbing habit is the most common. *Mondia* and *Tacazzea*, both African tropical and subtropical taxa, are lianas with stems up to 20 m long and 15 cm in diameter (Venter et al., 1990a; Venter, pers. obs. in *Mondia*) climbing into the canopies of riverine and swamp forest. However, climbers also occur in forest undergrowth, e.g., *Baroniella* in Madagascar (Klackenberg, 1997), and *Raphionacme flanaganii* Schltr. and *Ischnolepis natalensis* (Schltr.) Venter both from the Kwazulu-Natal Province in South Africa. Climbers are not restricted to moist environments, however. Some, like the two *Buckollia* species in northeastern Africa (Venter & Verhoeven, 1994b), inhabit dry savanna, others subdesert to desert scrub, e.g., *Cryptolepis volubilis* (Balf. f.) Schwartz on Socotra Island off the horn of Africa (Balfour, 1888) and *Periploca hydaspidis* Falc. of western Asia (Venter, 1997). Although less common, erect shrubs are found as well, e.g., *Cryptolepis stefaninii* Chiov. in northeastern Africa, the leafless *Periploca aphylla* Decne. and *P. visciformis* (Vatke) K. Schum in northeastern Africa and Arabia, *Finlaysonia curtisii* (King & Gamble) Venter in eastern Asia, *Ischnolepis graminifolia* (Costantin & Gallaud) Klack. in Madagascar (Klackenberg, 1999), and the three species of *Ectadium* in the Namib Desert in southwestern Africa (Venter et al., 1990b). Two small trees, *Cryptolepis arbuscula* (Radel.-Sm.) Venter (Radcliffe-Smith, 1971) and *Decalepis salicifolia* (Bedd. ex Benth. & Hook. f.) Venter (Beddome, 1876), are found on Socotra Island and in India, respectively. *Raphionacme* and *Schlechterella* are, in general, herbaceous, erect or climbing geophytes, and inhabit grassland, savanna, and dry scrub. Epiphytic taxa are rare and occur only in two genera, *Epistemma*, with three species, and the monotypic *Sarcorrhiza*, all of which have succulent stems, a unique feature in the subfamily (Bullock, 1962; Field & Hall, 1982; Huber, 1989). The two largest genera, *Cryptolepis* and *Raphionacme*, also exhibit the widest range of adaptations to different environments, from moist forest to savanna, semidesert scrub, true desert, and also grassland in the case of *Raphionacme*.

The stem typically has brownish to purplish bark, which may be glabrous, hairy, or verrucose. However, a few genera (e.g., *Batesanthus*, *Epistemma*, *Ischnolepis*, *Phyllanthera* Blume, and *Sarcorrhiza*) have a maroonish, grayish glossy, wax-like and flaky bark.

Leaves. The phyllotaxis of the leaves is opposite in all taxa, but in a few instances also fascicled, as in *Cryptolepis arbuscula* and *C. intricata* (Balf. f.) Venter of Socotra and *Ischnolepis* of Madagascar. Interpetiolar stipules occur (collar-like with frills in *Mondia* and collar-like with swollen teeth and reddish colleters in *Batesanthus* N. E. Br.), but in the majority of species only a line marks the position of the interpetiolar stipules. A large proportion of the taxa has colleters in the leaf axils. The petiole often has colleters that may also be scattered along the main vein of the lamina (e.g., *Tacazzea*). The

lamina is always simple and can get to be relatively large, as in *Batesanthus* (up to 16 × 11 cm), *Mondia* (up to 24 × 20 cm), *Omphalogonus* (up to 14 × 11 cm), and *Periploca nigrescens* Afz. (up to 16 × 11 cm), to small as in *Periploca aphylla* (3–12 × 1–5 mm, when its early deciduous leaves are present). The leaf lamina is variously shaped but mostly more or less ovate with an acute to cuspidate apex, margin always entire, surface usually glabrous, but may be hairy as in *Tacazzea*, even prickly (*Batesanthus talbotii* S. Moore), or it may have uniquely shaped, from round to elongated, hairy stomatal crypts spread over the lower surface (*Ectadium*; Venter et al., 1990b). The leaves are mostly mesomorphic and dorsiventral, but may be isobilateral in arid environments, for example in *Raphionacme hirsuta* where, in more favorable climates, the leaves are typically dorsiventral, but in arid areas exhibit isobilateral anatomy. A reddish mucro on the apical tip of the lamina is unique in a group of nine *Cryptolepis* species found in arid parts of Somalia in northeastern Africa, and Socotra Island and Yemen in Arabia (Venter & Verhoeven, 1999).

Inflorescences. Inflorescences are cymose and may be simple, bearing few flowers per inflorescence, or they may be compound dichasia with lateral monochasial branches and numerous flowers, or a cymose panicle with numerous flowers may occur as in *Myriopteron*, *Streptocaulon*, and *Tacazzea*. Paired bracts occur; mostly they are small and inconspicuous, but are sometimes conspicuous as in *Cryptolepis sanguinolenta* (Lindl.) Schltr. and *Telectadium dongnaiense* Pierre.

Flowers. Flower structure is highly complex. The presence of a corolline corona, corona-like nectaries, fusion of the stamens and style-head into a gynostegium, and pollen borne in tetrads or pollinia that are shed onto translators, all combine to create a flower adapted to complex animal pollination.

The flower is bisexual, actinomorphic, pentamerous (except for the bicarpellate gynoecium), and semi-epigynous. Flowers of different genera vary in length from about 6 cm in *Cryptostegia grandiflora* (Marohasy & Forster, 1991) to 3 mm in *Maclaudia felixii* Venter & R. L. Verh. (Venter & Verhoeven, 1994a) and 1 mm in *Finlaysonia wallichi* (Wight) Venter. The majority of taxa have flowers less than 10 mm long.

The *sepals* are usually uniform and inconspicuous, free or connate, glabrous or hairy, usually narrowly ovate to ovate or triangular, and green to purplish. In general the sepals have little taxonomic value, but are distinctive in a small number of taxa (e.g., *Zygostelma benthamii* Benth. with its unique obovate sepals and in *Raphionacme galpinii* Schltr. with its relatively long acicular sepals). Solitary or paired, inconspicuous, narrowly elliptic, elliptic or suborbicular colleters are inconsistently present between the sepals and petals, both solitary and paired colleters alternating with the sepals. The colleters are rarely conspicuous as in *Omphalogonus calophyllus* Baill., where they are relatively large and suborbicular in shape (Venter & Verhoeven, 1996a).

The *corolla* lobes are dextrorsely contorted in the bud. The only exception known is *Hemidesmus* R. Br., which has valvate aestivation (Endress & Bruyns, 2000). The corolla is differentiated into a lower and an upper tube. The lower corolla tube, which encircles the upper half of the semi-inferior ovaries, is composed of the congenitally fused products of the corolla, stamens, and nectaries. The upper corolla tube, in contrast, is formed through the postgenital fusion of just the corolla alone (see also Nishino, 1982; Sennblad et al., 1998, for additional details on congenital vs. postgenital corolla tube formation). The stamens and nectaries are thus epipetalous, and the lower tube cannot be receptacular in origin as alleged by Kunze (1990). The lower corolla tube is present in all taxa and is distinguished from the upper part of the corolla at the level of the nectaries. The remainder of the corolla above the lower tube may be fused into a distinct, shorter or longer, upper tube or the upper tube may be indistinct or very short with only the free corolla lobes above the nectary level.

A comparison of the floral structure in Periplocoideae with that of *Apocynum androsaemifolium* L. and *Parsonsia straminea* F. Muell. of the sister subfamily Apocynoideae reveals a similar lower corolla tube, but with the difference that it is composed of only the stamens and corolla tube. The nectary lobes are free, arising from the flower base.

Most of the genera in the Periplocoideae have an indistinct upper corolla tube, and the flowers are thus rotate or subrotate, e.g., in *Atherandra* Decne., *Finlaysonia* Wall., *Phyllanthera*, *Mondia*, *Periploca*, *Tacazzea*, etc. Genera with a broadly bowl-shaped upper tube belong to this group and result in a subrotate corolla as in *Epistemma* and *Finlaysonia curtisii* (King & Gamble) Venter. In those genera with a distinct upper corolla tube it may be campanulate as in *Cryptolepis* and *Raphionacme*, cylindrical as in *Gymnanthera*, or urceolate as in *Telectadium* Baill. and *Pentopetia urceolata* Klack. (Klackenberg, 1999). The corolla tube is rarely reflexed (turned inside out below the corolla lobe sinuses) as found in *Baseonema* Schltr. & Rendle and *Batesanthus* N. E. Br., the reflexion being more or

less level with the nectaries and the coronal annulus.

The inside of the lower tube is always glabrous, the upper tube is normally also glabrous, but it may be papillose (*Phyllanthera* and *Omphalogonus* Baill.) or hairy (*Pentopetia*). Vertical ridges ("staminal feet" of Endress & Bruyns, 2000) may run down the length of the tube as in *Raphionacme*. Vertical, groove-like cavities are unique in *Telectadium*. In *Cryptolepis*, *Cryptostegia*, *Gymnanthera*, *Pentopetia* (at least in *P. androsaemifolia* Decne. and *P. albicans* (Jum. & H. Perrier) Klack.), *Raphionacme*, and *Telectadium* shallow or deep nectary pockets are present in the corolla just above the nectaries, which in these genera are not lobular but shelf-like and often small.

Corolla lobes are usually glabrous adaxially, but they may be hairy (*Finlaysonia*, *Periploca*, and *Pentopetia*), darkly spotted and/or with coronal swelling (*Buckollia* and *Sarcorrhiza*) or with a coronal trough (*Periploca*) near their inner bases or, rarely, the adaxial surface may be papillose (*Omphalogonus* and *Phyllanthera*). The petals are white, green, creamy-yellow, pinkish, maroon, purple, blue, yellow, orange, or red, the last three colors more common in Madagascar and Asia.

The *corona* consists of corolline lobes, outgrowths of the corolla tube alternating with the corolla lobes. Kunze (1990) regarded these corona lobes as having been evolved from colleters at the inner base of the petals. If true, a second set of corona lobes, found in the petal sinuses of *Cryptolepis oblongifolia* (Meisn.) Schltr. and *C. producta* N. E. Br., is unlikely to have evolved from colleters. If the corolla itself has the potential to initiate this second set of corona lobes, could the same not be true of the first set?

The corona may arise at various positions inside the corolla tube, but, as stated above, always alternate to the corolla lobes. This contrasts, for example, with *Apocynum androsaemifolium* of the Apocynoideae where the corona lobes are borne opposite the petals. In Periplocoideae, the corona lobes, furthermore, are always borne above the stamens, whether directly above them or distanced from them. In rotate flowers with an indistinct upper corolla tube, such as in *Atherandra*, *Decalepis* Wight & Arn., *Finlaysonia*, *Hemidesmus*, *Mondia*, *Periploca*, and in subrotate flowers with a broadly bowl-shaped upper corolla tube, such as in *Epistemma*, *Finlaysonia curtisii*, and *Zygostelma* Benth., the corona lobes arise directly above the stamens, which are nearly level with the nectaries at the apex of the lower corolla tube. In *Cryptolepis*, *Omphalogonus*, and *Telectadium* the corona lobes are borne toward the middle of a distinct upper corolla tube, or in *Ectadium*, *Gymnanthera*, *Maclaudia* Venter & R. L. Verh., and *Raphionacme* the corona lobes arise at the mouth of a distinct corolla tube. *Pentopetia* and *Stomatostemma* N. E. Br. have their corona lobes in the petal sinuses. The corona may be concealed within the corolla tube (e.g., *Cryptolepis* and *Cryptostegia*) or it may be exserted from it (*Gymnanthera*, *Raphionacme*, and *Schlechterella*).

Corona lobe form varies among genera and even among species within genera (Venter et al., 1990a); it may be entire (*Tacazzea*), bisegmented (*Periploca*), trisegmented (*Periploca*, *Raphionacme*), or tripartite (*Raphionacme*). Its shape may be cordate to reniform (*Mondia*), ovate (*Finlaysonia khasiana* (Kurz) Venter, *Tacazzea rosmarinifolia* (Decne.) N. E. Br.) to suborbicular (*Decalepis*), linear (*Sacleuxia*, *Periploca gracilis* Boiss.), filiform (*Atherandra*, *Ischnolepis*, *Myriopteron*, *Streptocaulon*, *Tacazzea*), club-shaped (*Cryptolepis*), match-like (*Stomatostemma*), or radiating ridges (*Omphalogonus*, *Telectadium*). The apex of the corona lobe may be entire (*Ischnolepis*, *Pentopetia*, *Sarcorrhiza*), bifid (*Periploca gracilis*, *Sacleuxia*), or variously branched (both *Schlechterella* species, *Periploca nigrescens* Afzel.). The most common type of lobe found in a large proportion of the taxa is the simple filiform type.

The corona lobes are variously colored, often of a color contrasting with that of the corolla lobes, for instance purple corona lobes and bright green corolla lobes as in *Raphionacme velutina* Schltr. The corona lobes are, furthermore, mostly glabrous, but may be papillose (e.g., *Periploca angustifolia* Labill.) or hairy (e.g., *Pentopetia dasynema* Choux and *Periploca hydaspidis* Falc.). The corona lobes may also be much reduced or absent as in *Baroniella*, *Baseonema*, *Batesanthus*, and *Phyllanthera*.

In most of the taxa the corona lobes are free from one another, but they may be fused together in an annulus outside the stamens as in *Baroniella* and *Camptocarpus* (Klackenberg, 1997, 1998) and in a few species of *Raphionacme* (*R. excisa* Schltr., *R. kubangensis* S. Moore, and *R. utilis* N. E. Br.).

As stated above, an additional corolline corona may, in rare cases, be present outside and above the corona. In *Cryptolepis oblongifolia* and *C. producta*, in addition to the typical corona lobes placed lower down in the corolla tube, pockets (fusion of the corolla tube with the basal adaxial faces of two adjacent petals) sometimes occur at the petal sinuses, and short filiform corona lobes inconsistently arise from the apical margins of these sinus pockets.

The *stamens* arise opposite the sepals and are

always inserted below the outer corona lobes, at various positions within the corolla tube (Endress & Bruyns, 2000, Figs. 2–7). Stamen insertion on the corolla, as compared with corona insertion, is an important diagnostic character in the Periplocoideae. In rotate flowers with an indistinct upper corolla tube both stamens and corona are located just above the level of the nectaries (e.g., *Atherandra*, *Mondia*, *Myriopteron*, *Tacazzea*, and *Zygostelma*). In genera with a distinct upper corolla tube stamens and corona may both occur at the mouth (*Gymnanthera* and *Raphionacme* (Endress & Bruyns, 2000, Figs. 2, 5), and *Schlechterella*) or the stamens may be near or at the base of the upper corolla tube and the corona around the middle of the tube (*Cryptolepis* (Endress & Bruyns, 2000, Fig. 6C, D)), or at the mouth (*Ectadium* (Endress & Bruyns, 2000, Fig. 6A, B) and *Maclaudia* (Venter & Verhoeven, 1994a)), or in the sinuses (*Pentopetia* (Klackenberg, 1999, Fig. 8) or *Stomatostemma* (Venter & Verhoeven, 1993, Fig. 10B, or Endress & Bruyns, 2000, Fig. 7A, B)). However, *Pentopetia* is an exception to the rule: in all of its 21 species the corona lobes consistently arise in the petal sinuses, but the depth of the upper corolla tube varies from indistinct to distinct with the stamens arising from the level of the nectaries to in the mouth of the upper corolla tube (Klackenberg, 1999).

The stamens are simple, consisting of filament and anther, the latter, in all but one genus, being fused with the style-head. The exception is *Hemidesmus*, in which the filaments are fused to the style-head and the anthers thus elevated above the style-head. The anthers always connive over the style-head with their connective apices touching, seldom agglutinated, as in *Decalepis* and *Hemidesmus*. An anther comprises two anther locules, each with two thecae, in which pollen is borne in tetrads or in pollinia. In contrast to the Apocynoideae and Asclepiadoideae no lignified tissue occurs in the margins of the anthers, nor do the anthers extend into a swallow-tailed base, and thus no guide rails are present in the Periplocoideae (Endress & Bruyns, 2000). The anther connective extends beyond the thecae as an apical appendage. This appendage is small and lobular or deltoid in most of the genera (e.g., *Cryptolepis*, *Periploca*, *Raphionacme*, *Streptocaulon*), but it may be enlarged into colorful oblong (*Finlaysonia khasiana* and *F. obovata* Wall.), lance-like (*Atherandra acuminata* Decne.), or foliar (*Phyllanthera* Blume) bodies (Schumann, 1895, Fig. 63A, B, D). The anther varies in shape from ovate to narrowly triangular in most of the taxa, but may be hastate (*Cryptostegia*), oblong (*Atherandra*), or depressed ovate (*Mondia*). In a few taxa, e.g., *Cryptostegia*, *Raphionacme grandiflora* N. E. Br., and *Telectadium*, callosities occur at the anther bases. Hairy anthers are diagnostic for *Ectadium*, *Pentopetia*, and *Periploca*. Glabrous anthers are typical for most of the 27 species of *Cryptolepis*, but hairy anthers do occur in *C. eburnea* (Pichon) Venter and occasionally in *C. stefaninii* Chiov. In *Omphalogonus* the anthers are also occasionally hairy.

In the majority of the genera (with the exceptions of *Baroniella* and *Camptocarpus*), in all taxa where the corona is not distanced from the stamens, the bases of the staminal filaments are fused outwardly with the corona lobe bases to create staminal feet (Endress & Bruyns, 2000, Figs. 2, 3, 4; Forster, 1989, Fig. 7) These staminal feet may be fused laterally with one another into an annulus outside the nectaries (Forster, 1993, Fig. 1), or the staminal feet may be fused laterally with the nectaries as in *Baseonema* and *Tacazzea*. In *Hemidesmus* a unique fusion of the staminal feet, staminal filaments, nectary lobes, and style occurs, creating a closed tube around the style with 5 nectary pockets on the outside of the tube (Endress & Bruyns, 2000, Fig. 6H). Another unique fusion of the stamens with the nectaries is found in *Phyllanthera* (e.g., *Phyllanthera grayi* (P. I. Forst.) Venter; Forster, 1990, Fig. 3). Superficially this fusion resembles that found in *Hemidesmus*, but there are differences. In *Phyllanthera* the nectary lobes are fused laterally into a tube, but the fusion is incomplete, as 5 slits are left opposite the staminal feet. This tube is, furthermore, fused at its apex to the style and at its bottom to the staminal feet, but not to the staminal filaments, as found in *Hemidesmus*. The end result of this fusion is a conical nectary tube around the style, with 5 apertures that give access to the cavity between style and nectary tube. In contrast to *Hemidesmus*, and the rest of the Periplocoideae, where the nectar is exposed, in *Phyllanthera* there is no indication that nectar is produced on the outside of the nectary tube. Rather it is produced inside the nectary tube and must be collected from the gynoecial cavity through the slits in the nectary tube.

Staminal coronal appendages, as can be observed in the Asclepiadoideae, do not occur in the Periplocoideae, although fusion of the staminal filaments with the corona lobes and sometimes also the nectary lobes may result in staminal feet, which may be oblong (*Raphionacme excisa*) or lobular to deltoid (*Finlaysonia obovata* Wall.; Forster, 1990, Fig. 3) in shape, and which may be interpreted as a type of staminal corona.

Pollen tetrads are found in 24 of the Periplocoi-

deae genera, representing 157 (87%) of the species, as compared to 7 genera with 24 species (13%) with pollinia (Verhoeven & Venter, 1998, 2001 this volume). Of the genera with pollinia, 5 (*Decalepis*, *Finlaysonia*, *Gymnanthera*, *Hemidesmus*, and *Streptocaulon*) are from Asia and Australia. The other 2, *Epistemma* and *Schlechterella*, are restricted to Africa. In Madagascar and the Mascarene Islands no genera with pollinia are found. The pollinia (Venter & Verhoeven, 1998) are elongate or more or less round in shape and are loosely agglutinated. Both tetrads and pollinia have 3 to 6 pores per grain throughout the subfamily, except in *Baseonema* (tetrads), *Raphionacme* (tetrads), and *Schlechterella* (pollinia) where 8 to 16 pores per grain occur.

Interstaminal, antepetalous lobes or nectaries (Kunze, 1990) arise from the apex of the lower corolla tube. These *nectaries* may be conspicuous lobes alternating with the stamens as in *Tacazzea pedicellata* K. Schum. where they encircle the style, or they may be shelf-like or rim-like and less conspicuous as in *Cryptolepis* and *Raphionacme*, or they may be tubular around the style as in *Hemidesmus* (also see nectary pockets, character 33, Appendix 1). In all genera with rotate or subrotate flowers where the upper corolla tube is indistinct or at most bowl-shaped and the stamens exposed, the nectary lobes are lobular (although differently shaped in different taxa) and fused laterally to the staminal feet. Twenty-one of the 31 genera of the Periplocoideae fit this description—all of them in the *Atherandera*-clade, except for *Pentopetia*. In the group of genera with a distinct upper corolla tube two conditions occur as far as the nectaries are concerned. In that group where the stamens are present in the corolla tube mouth or exserted from it—the *Gymnanthera*-clade (*Gymnanthera*, *Raphionacme*, and *Sclechterella*)—the nectaries have not migrated with the stamens (and corona) to the corolla tube mouth, but can be seen as nectary shelves at the apex of the lower corolla tube (Endress & Bruyns, 2000, Fig. 3C just above the ovary). In the second group of genera (*Cryptolepis*, *Cryptostegia*, *Ectadium*, *Maclaudia*, *Omphalogonus*, *Stomatostemma*, and *Telectadium*) the stamens are more or less level with the nectaries, which are also shelf-like (Endress & Bruyns, 2000, Fig. 6B, E, alternating with the staminal feet encircling the top half of the ovaries).

The *gynoecium* is apocarpous and consists of two subspherical, semi-inferior ovaries, the two styles of which unite further up into one compound style that terminates in a dilated style-head (stigmatic head). The ovaries are glabrous in all taxa except in *Sacleuxia tuberosa* (E. A. Bruce) Bullock, in which they are pubescent. The style(s) may vary in length from nearly absent in *Cryptolepis*, *Cryptostegia*, and *Decalepis* to relatively long in *Gymnanthera*, *Phyllanthera*, and *Raphionacme*, and stylar fusion may begin just above the ovaries or closer to the style-head. The compound style is uniformly terete and glabrous, except in *Raphionacme excisa* Schltr. where it is furrowed and hairy. The style-head is more or less pentagonal in cross section, and three zones are discernable, namely upper, marginal, and lower zones. Apart from being pentagonal, the style-head varies considerably in shape, size, and length, even within genera. The style-head is normally broadly ovoid to ovoid or conical, but may be cylindrical as in *Raphionacme excisa*, very narrowly conical as in *Atherandra*, or obconical as in *Decalepis*. The apex of the style-head is normally acute or round, but it may be flattened (*Decalepis*) or bifid (*Periploca somaliense*).

The *translators*, which are formed of hardened secretions of the style-head, are embedded in its upper surface and margin. Translators are basically spathulate in shape with a sticky disc (viscidium) (on the lower zone of the style-head), a stipe (on margin and upper zone of the style-head), and a scoop-like receptacle, onto which the pollen is shed (on the upper zone of the style-head) (Verhoeven & Venter, 1997). Five translators are secreted per style-head, and they alternate with the anthers (Endress & Bruyns, 2000, Fig. 6G) in such a way that the pollen tetrads or pollinia from two adjacent anther locules are shed onto the same translator receptacle. The translators, in particular the receptacles, vary considerably in shape from elliptic to narrowly elliptic with virtually no stipe present (e.g., *Baroniella*, *Cryptolepis*, *Ectadium*, and *Ischnolepis* (Endress & Bruyns, 2000, Fig. 3E, F)) to rhomboid (*Tacazzea* (Venter et al., 1990a, Fig. 11)) or with distinct stipe (*Raphionacme* (Verhoeven & Venter, 1997, Figs. 17–10)). The translators of the genera with pollinia are more complex in structure and shape with the receptacle hollow. The translator of *Hemidesmus* is unique in being bent z-shaped with a cup-like receptacle into which the pollinia snugly fit (Endress & Bruyns, 2000, Fig. 6I). In *Decalepis* the translator receptacles resemble the hollow receptacle of a spoon, and these fit into five hemispherical hollows on the flat upper zone of the style-head. Swarupanandan et al. (1996) regarded gynoecium structure as important for higher level classification in the Asclepiadoideae (Periplocoideae excluded), but as far as the Periplocoideae are concerned, the gynoecium is, in general, too variable within a genus to be of importance in classification above species level.

At the generic level the position of the gynostegium in relation to the corolla is more consistent than that of the pistil. In flowers that are rotate or subrotate (e.g., *Finlaysonia*, *Mondia*, *Periploca*, *Streptocaulon*, *Tacazzea*, etc.) the upper corolla tube is virtually absent. In these types of flower and in flowers with a bowl-shaped upper corolla tube the gynostegium is completely exposed above the corolla. In those taxa with a distinct campanulate, trumpet-shaped, cylindrical, or urceolate upper corolla tube the gynostegium is concealed deep within the tube as in *Ectadium*, *Cryptolepis*, *Cryptostegia*, and *Telectadium*, or the gynostegium is situated in the corolla tube mouth or is exserted from it as in *Gymnanthera*, *Raphionacme*, and *Schlechterella*.

The follicles are usually paired, although only one may develop as is the case in *Raphionacme galpinii* Schltr. and *R. palustris* Venter & R. L. Verh. (Venter & Verhoeven, 1986a). The follicles are mostly divergent and may be erect or pendulous, variably so within genera. The follicles are mostly narrowly ovoid (cigar-shaped) with an acute to acuminate apex, but may be narrowly cylindrical (*Periploca graeca* L.), narrowly cylindrical with sinuate margins (*Baroniella*, *Camptocarpus*), ovoid with a long apiculate apex (*Tacazzea apiculata* Oliv.), keel-shaped (*Raphionacme namibiana* Venter & R. L. Verh. and *Finlaysonia obovata*), or with unique longitudinal membranous wings (as in *Myriopteron*). However, follicle shape may be variable within genera, e.g., *Tacazzea apiculata* (apiculate) and *T. conferta* (cigar-shaped).

Each follicle contains numerous seeds. The seed structure is rather uniform, being compressed, mostly obliquely ovate to oblong-ovate to elliptic with a micropylar coma of silky hairs. The seeds are thus adapted to wind dispersal, floating on air with the aid of the coma. There are two known exceptions. One is *Raphionacme namibiana* of the semi-desert of southwestern Africa, which has a peripheral ring of hairs (Venter & Verhoeven, 1986b) that do not seem to be an adaptation to floating in the air, but perhaps to be blown along the ground surface. The other exception is the Asian *Finlaysonia obovata*, which inhabits mangrove swamps. The seeds of this species have annular membranous wings with hairy margins, which possibly let the seeds float on water; they are also the largest seeds in the subfamily.

Phylogenetic Analysis

Parsimony analysis yielded 80 shortest trees, each 154 steps, consistency index (CI) = 0.51, retention index (RI) = 0.62. The strict consensus tree based on the data matrix (Table 1), consisting of 33 taxa and 39 characters (Appendix 1), is shown in Figure 1. The ingroup is well supported as monophyletic with bootstrap and decay values of 91% and > 1, respectively. There is support of > 50% for three clades.

The first, the *Atherandra*-clade (denoted by A in Fig. 1), has a bootstrap support of 69%, and is characterized by a gynostegium that is exposed from the corolla (character 38). Within this clade, *Tacazzea* and *Mondia* are successive sisters to the remainder, which form a polytomy of 18 genera within which two subclades, neither with support > 50%, are recognizable. The *Baroniella*-subclade of only *Baroniella* and *Camptocarpus*, both endemic to Madagascar and the Mascarenes, are distinguished by having their corona lobes fused into an annulus around and outside the stamens (character 16).

The second subclade divides into two smaller subclades. The first of these is comprised of the sisters *Baseonema* and *Batesanthus*, two African genera characterized by the absence of corona lobes (character 18) and the presence of a reflexed corolla (character 10) with 95% support, and these subtended by *Phyllanthera*, but this genus supported by < 50%. The inclusion of the Asian *Phyllanthera* was unexpected, but may be supported by the absence of corona lobes (character 18) and same type of bark (character 5). The second smaller subclade of *Epistemma*, *Ischnolepis*, *Sacleuxia*, and *Sarcorrhiza*—found in Africa (*Ischnolepis* also in Madagascar)—has < 50% support, and these genera are characterized by the presence of tubers (character 2) and a maroonish grayish, glossy, flaky stem bark (character 5).

The second clade, with > 50% bootstrap support, is the *Cryptolepis*-clade (denoted by B in Fig. 1), having a bootstrap value of 66%. This clade is comprised of only *Cryptolepis* and *Cryptostegia*. These two genera are characterized by having a distinct corolla tube (character 9), their gynostegia are concealed in the corolla tube (character 38), and a unique swelling between stamens and corona lobes is common (character 20). Successive sister genera to this clade are *Maclaudia*, *Ectadium*, and *Stomatostemma*, which have a distinct corolla tube (character 9), gynostegium concealed in the corolla tube (character 38), and corona lobes arising at the corolla mouth or in the corolla lobe sinuses (character 13).

The third clade (denoted by C in Fig. 1), with > 50% bootstrap support, includes three genera: the sisters *Raphionacme* and *Schlechterella*, supported by 97%, and these, together with their successive

Table 1. Data matrix for cladistic analysis of the Periplocoideae. (Characters = 1–39; character states = 0–5; ? = character state inapplicable; outgroup genera = *Parsonsia* and *Apocynum*.)

Atherandra	000000040000020021000001110020110022010
Baroniella	101000040000020200000010010020100011011
Baseonema	000100030100020030000000010021110021010
Batesanthus	000110030100020030000000010020110021010
Buckollia	000000040001020011000000010020110021010
Camptocarpus	101000140000020111000100010020110021011
Cryptolepis	000000001000100001210010010020111110000
Cryptostegia	000000011000310001410010010020111110000
Decalepis	012000040000020022000020010040110022010
Ectadium	200000011000210001000010111120110010000
Epistemma	300011040010020011300000010040110011010
Finlaysonia	000000040000020021000010010030110022010
Gymnanthera	000000011000210011001020010040101123130
Hemidesmus	000000040000020021000030010040122223020
Ischnolepis	212010040000020011000000010020110021010
Maclaudia	000000201000210001000010010020110010000
Mondia	012200240000020012000100110020110010010
Myriopteron	000000240000020021000000010020110021010
Omphalogonus	000000001020100002400000011120111121000
Pentopetia	100000001010310001000000010120100021030
Periploca	000000040012021011000010011020110021010
Phyllanthera	000010040020020030?000312100201303220 20
Raphionacme	423000001000210011001000010021101121130
Sacleuxia	212100040000020011400100010020100021010
Sarcorrhiza	312011040001020011000000010020110021010
Schlechterella	123000001000210112501000010031101121130
Stomatostemma	000000201000310001300000010020111111000
Streptocaulon	000000240000020021000100010030110022010
Tacazzea	000000240000020011400010010020110010010
Telectadium	200000021010100001001000010020101121000
Zygostelma	000000040000021021000010010020110022010
Parsonsia	000000001000?????2000000000000000?000
Apocynum	200000001000100000000000000001000000?000

sister, *Gymnanthera*, with a support of 83%. The genera in this clade have a distinct corolla tube (character 9), which may be campanulate or trumpet-shaped (character 8), and corona lobes and gynostegium in the mouth of the corolla tube (characters 13 and 37). The remaining genera, *Omphalogonus*, *Pentopetia*, and *Telectadium*, are unresolved.

Discussion and Conclusions

Even though the Periplocoideae are a relatively small entity as far as number of taxa is concerned, they occur widespread over a considerable part of the world in a variety of niches, in swamp forest, rainforest, mangrove forest, savannna, grassland, semi-desert, and desert scrub, and exhibit a variety of growth forms, from lianas to shrubs and trees to suffrutescent climbers and herbaceous geophytes to epiphytes. The flower is complex, adapted to animal pollination through a variety of corolla forms, degrees of corolla fusion, presence of variously structured corolline coronas, and morphological synorganizations and fusions between the corona and parts of the androecium, formation of a gynostegium, intricate pollen translators, and the formation of pollen tetrads or pollinia. The seeds of the Periplocoideae are, with perhaps one exception, adapted to wind dispersal.

N. E. Brown (1902, 1904) placed much emphasis on the distinctness or indistinctness of the corolla tube in separating the genera of the Periplocoideae. Venter and Verhoeven (1997) followed his example and based their tribal classification on the absence or presence of the upper corolla tube. However, this assumption proved to be wrong, and it eventually became clear that the corolla is much more variable within a genus than previously surmised. The corolla tube may be distinct in some species, but in-

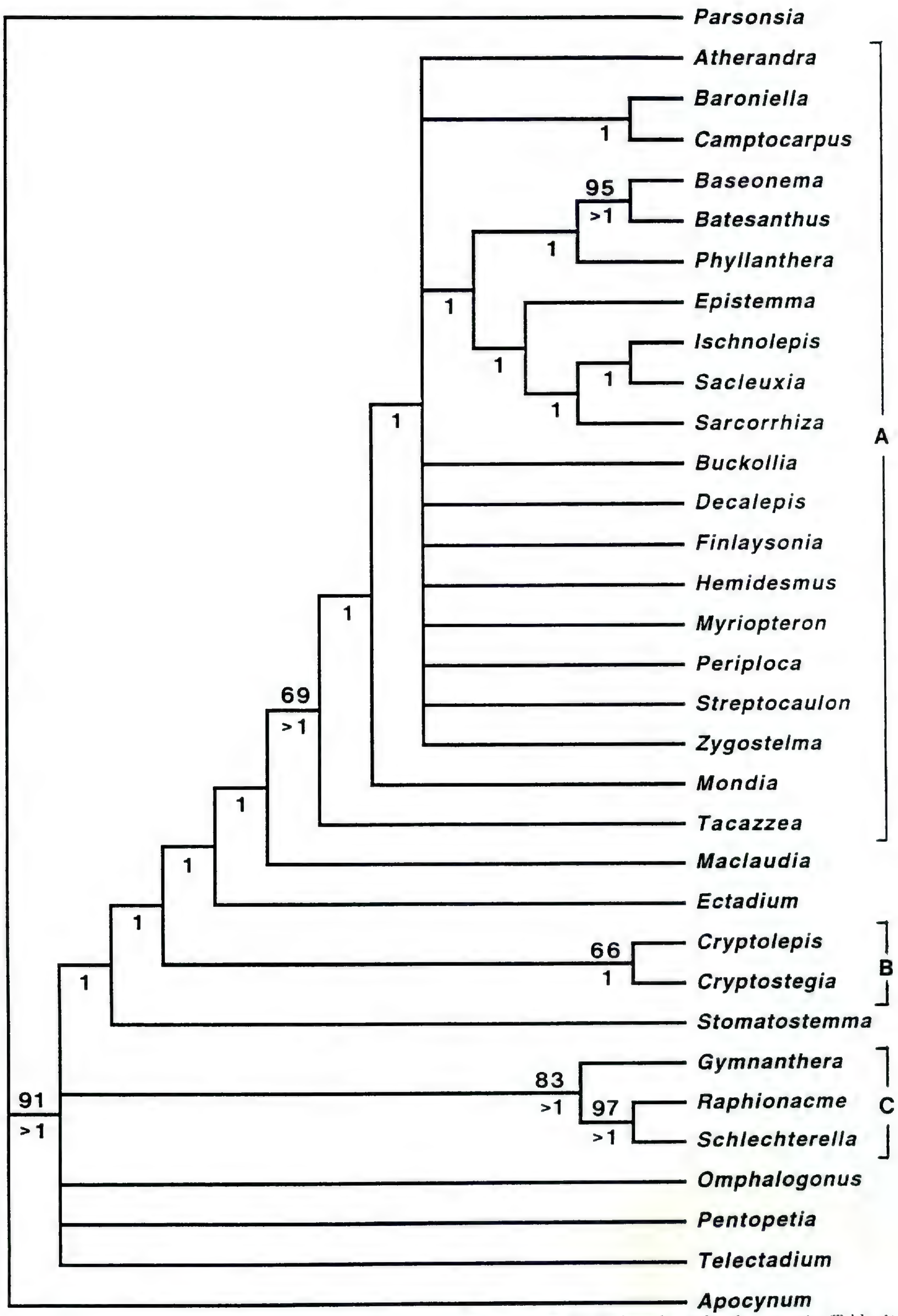


Figure 1. Strict consensus tree of the Periplocoideae genera using PAUP, based on the data matrix (Table 1), consisting of 33 taxa and 39 characters. Length of most parsimonious trees = 154; number of most parsimonious trees = 80; consistency index (CI) = 0.51; retention index (RI) = 0.62.

distinct in other species of the same genus. The Madagascan endemic, *Pentopetia* with 21 species (Klackenberg, 1999), and the much smaller *Schlechterella* of eastern Africa with 2 species (Venter & Verhoeven, 1998), in particular, illustrate this phenomenon.

In the phylogenetic analysis presented here, the monophyly of the Periplocoideae is well supported, but relationships among the 31 genera are, for the most part, poorly resolved. Three clades are relatively well supported, the *Atherandra*-clade, including 20 genera, the *Cryptolepis*-clade, with 2 genera, and the *Gymnanthera*-clade, with 3 genera. The placement of the remainder of the genera is tenuous. The three tribes as delimited by Venter and Verhoeven (1997) are not supported.

Despite the generally poor support values, the phylogenetic analysis presented here (Fig. 1) provides a platform upon which future studies in the subfamily may build. One of the exciting results of our analysis is that it suggests that pollinia have probably evolved more than once in the Periplocoideae. Five of the genera with pollinia (*Decalepis*, *Epistemma*, *Finlaysonia*, *Hemidesmus*, and *Streptocaulon*) belong to the *Atherandra*-clade, whereas *Gymnanthera* and *Schlechterella* belong to the *Gymnanthera*-clade. Parallel evolution of pollinia in two clades of Periplocoideae is also supported by distinct differences in the pollen grain apertures in the two groups (Verhoeven & Venter, 2001), and questions the homology of pollinia in Secamonoideae and Asclepiadaceae.

As stated above, the Periplocoideae are a diverse and complex group of taxa, which led to the description of a large number of monotypic or small genera by a variety of botanists from different countries in the previous three centuries. This is especially true of the Asian part of the subfamily. The present revision of the Periplocoideae is the first to be done on a global scale. For the first time all taxa could be compared and their relationships studied. As a consequence, this required a number of synonymies of genera and species, and also new combinations, the newest of which are presented in Appendices 2 and 3 herein. The key to the genera, presented in Appendix 4, is also the first global key for the Periplocoideae.

Literature Cited

Balfour, I. B. 1888. Botany of Socotra. Asclepiadaceae. Trans. Roy. Soc. Edinburgh 16: 162–167.

Beddome, R. H. 1876. *Utleria*. *In* G. Bentham & J. D. Hooker, Genera Plantarum 2: 743. London.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795–803.

Brown, N. E. 1902. Asclepiadeae. *In* W. T. Thiselton-Dyer, Flora of Tropical Africa 4: 233–234. Lovell Reeve, London.

———. 1904. Asclepiadeae. *In* W. T. Thiselton-Dyer, Flora Capensis 4: 520. Lovell Reeve, London.

Bullock, A. A. 1955. Notes on African Asclepiadaceae, No. 4. Kew Bull. 10: 358–359.

———. 1962. *Sarcorrhiza epiphytica*. *In* Hooker's Icon. pl. 36: t 3585.

Catalan, M. P., E. A. Kellogg & R. G. Olmstead. 1997. Phylogeny of Poaceae, subfamily Pooideae based on chloroplast *ndh*F gene sequences. Molec. Phylogenet. Evol. 8: 150–166.

Donoghue, M. J., R. G. Olmstead, J. F. Smith & J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Ann. Missouri Bot. Gard. 79: 333–345.

Endress, M. E. & P. V. Bruyns. 2000. A revised classification of the Apocynaceae, s.l. Bot. Rev. (Lancaster) 66: 1–56.

Field, D. V. & J. B. Hall. 1982. *Epistemma*, a new genus of Periplocaceae from West Africa. Kew Bull. 37: 117–120.

Forster, P. I. 1989. Notes on Asclepiadaceae, 1. Austrobaileya 3: 109–133.

———. 1990. Notes on Asclepiadaceae, 2. Austrobaileya 3: 273–289.

———. 1991. A taxonomic revision of *Gymnanthera* R. Br. (Asclepiadaceae: Periplocoideae) in Australia. Austral. Syst. Bot. 4: 563–569.

———. 1993. Conspectus of *Cryptolepis* R. Br. (Asclepiadaceae: Periplocoideae) in Maleisia. Austrobaileya 4: 67–73.

Grellier, P., L. Ramiararnanana, V. Millerious, E. Deharo, J. Schrevel, F. Frappier, F. Trigalo, B. Bodo & J. L. Pousset. 1996. Antimalarial activity of cryptolepine and isocryptolepine, alkaloids isolated from *Cryptolepis sanguinolenta*. Phytotherapy Res. 10: 317–321.

Huber, H. 1989. Trois nouvelles espèces d'Asclepiadales du Cameroun. Bull. Mus. Natl. Hist. Nat., B, Adansonia 11: 447–452.

Hutchings, A., A. H. Scott, G. Lewis & A. B. Cunningham. 1996. Zulu Medicinal Plants. Univ. Natal Press, Pietermaritzburg.

Katz-Downie, D. S., C. M. Valiejo-Roman, E. I. Terentieva, A. V. Troitsky, M. G. Pimenov, B. Lee & S. R. Downie. 1999. Towards a molecular phylogeny of Apiaceae, subfamily Apioideae: Additional information from nuclear ribosomal DNA ITS sequences. Pl. Syst. Evol. 216: 167–195.

Klackenberg, J. 1997. Revision of the genus *Baroniella* Costantin & Gallaud (Asclepiadaceae, Periplocoideae). Candollea 52: 383–407.

———. 1998. Taxonomy and phylogeny of the genus *Camptocarpus* s.l. (Periplocoideae, Asclepiadaceae). Bot. Jahrb. Syst. 120: 45–85.

———. 1999. Revision of the Malagasy genera *Pentopetia* and *Ischnolepis* (Apocynaceae s.l., Periplocoideae). Candollea 54: 257–339.

Kunze, H. 1990. Morphology and evolution of the corona in Asclepiadaceae and related families. Trop. Subtrop. Pflanzenwelt 76: 1–51.

Marohasy, J. & P. I. Forster. 1991. A taxonomic revision of *Cryptostegia* R. Br. (Asclepiadaceae: Periplocoideae). Austral. Syst. Bot. 4: 571–577.

Nilsson, S., M. E. Endress & E. Grafström. 1993. On the

relationship of the Apocynaceae and Periplocaceae. Grana, Suppl. 2: 3–20.
Nishino, E. 1982. Corolla tube formation in six species of Apocynaceae. Bot. Mag. (Tokyo) 95: 1–17.
Potgieter, K. & V. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.
Radcliffe-Smith, A. 1971. *Mitolepis arbuscula*. *In* Hooker's Icon. pl. 37: t 3876.
Schumann, K. 1895. Asclepiadaceae. *In* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien 4(2): 189–306. Leipzig.
Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.
——— & ———. 2000. Is there a justification for differential a priori weighting in coding sequences? A case study from *rbc*L and Apocynaceae s.l. Syst. Biol. 49: 101–113.
———, M. E. Endress & B. Bremer. 1998. Morphology and molecular data in phylogenetic fraternity—The tribe Wrightieae (Apocynaceae) revisited. Amer. J. Bot. 85: 1143–1158.
Stafleu, F. A. & R. S. Cowan. 1976. Taxonomic Literature, Vol. 1. Bohn, Scheltema & Holkema, Utrecht.
——— & ———. 1979. Taxonomic Literature, Vol. 2. Bohn, Scheltema & Holkema, Utrecht.
——— & ———. 1983. Taxonomic Literature, Vol. 4. Bohn, Scheltema & Holkema, Utrecht.
Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996. The subfamilial and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.
Swofford, D. L. 1998. PAUP*, Phylogenic Analysis Using Parsimony (*and other methods), Vers. 4. Sinauer, Sunderland, Massachusetts.
Venter, H. J. T. 1997. A revision of *Periploca* (Periplocaceae). S. African J. Bot. 63: 123–128.
——— & R. L. Verhoeven. 1986a. A new species of *Raphionacme* (Periplocaceae) from Natal, South Africa. S. African J. Bot. 52: 149–152.
——— & ———. 1986b. A new species of *Raphionacme* (Periplocaceae) from South West Africa/Namibia. S. African J. Bot. 52: 332–334.
——— & ———. 1993. A taxonomic account of *Stomatostemma* (Periplocaceae). S. African J. Bot. 59: 50–56.
——— & ———. 1994a. *Maclaudia felixii*, a new genus and species in the Periplocaceae from Guinea, West Africa. Bot. J. Linn. Soc. 115: 57–63.
——— & ———. 1994b. *Buckollia*, a new genus in the Periplocaceae. S. African J. Bot. 60: 93–98.
——— & ———. 1996a. The "minor confusion" around *Parquetina nigrescens* (Periplocaceae). S. African J. Bot. 62: 23–30.
——— & ———. 1996b. *Raphionacme haeneliae* (Periplocaceae), a new species from the Namib Desert, Namibia. S. African J. Bot. 62: 316–320.
——— & ———. 1997. A tribal classification of the Periplocoideae (Apocynaceae). Taxon 46: 705–720.
——— & ———. 1998. A taxonomic revision of *Schlechterella* (Periplocoideae, Apocynaceae). S. African J. Bot. 64: 350–355.
——— & ———. 1999. A new species of *Cryptolepis* (Periplocoideae, Apocynaceae) from Arabia. Bot. J. Linn. Soc. 131: 417–422.
——— & ———. 2000. *Raphionacme sylvicola* (Apocynaceae, Periplocoideae), a new species from Zambia. Novon 10: 169–174.
———, ——— & J. D. S. Kotze. 1990a. A monograph of *Tacazzea* (Periplocaceae). S. African J. Bot. 56: 93–112.
———, ——— & ———. 1990b. A taxonomic revision of *Ectadium* (Periplocaceae). S. African J. Bot. 56: 113–124.
Verhoeven, R. L. & H. J. T. Venter. 1997. The translator in *Raphionacme* (Periplocaceae). S. African J. Bot. 63: 46–54.
——— & ———. 1998. Pollinium structure in Periplocoideae (Apocynaceae). Grana 37: 1–14.
——— & ———. 2001. Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 569–582.

Appendix 1.

Characters and Character States

Vegetative:

1. Habit
   0—perennial climbers
   1—suffrutescent climbers
   2—erect shrubs or trees
   3—epiphytes
   4—erect herbs
2. Tuber presence and shape
   0—absent
   1—present and cylindrical to subspherical
   2—present and turnip-shaped
3. Growth mode
   0—perennial without tubers
   1—suffrutescent without tubers
   2—perennial with tubers
   3—suffrutescent from a tuber
4. Interpetiolar stipules
   0—interpetiolar line
   1—interpetiolar collar inflated and dentate
   2—interpetiolar collar with frills

Stipules or outgrowths in the region where stipules would normally occur are common in several families of the Gentianales. In the Apocynaceae, the most common type of outgrowth is just a line or ridge between the petioles, and is thus often called an interpetiolar ridge or line, and is generally assumed to be homologous to stipules.

5. Stem bark
   0—bark brownish or purplish and glabrous, hairy, and/or verrucose
   1—bark maroonish grayish, glossy, wax-like, and flaky
6. Stem succulence
   0—stems not succulent
   1—stems succulent

Inflorescence:

7. 0—inflorescence with 1 to 10 flowers and racemose-like
   1—inflorescence 1- to 10-flowered and umbel-like
   2—inflorescence many flowered and panicle-like

Corolla

8. Corolla shape

0—campanulate
1—trumpet-shaped
2—urceolate
3—reflexed
4—rotate

9. Corolla fusion
0—corolla fused up to the nectary level
1—corolla fused to above the nectary level

10. Corolla tube reflexion
0—tube not reflexed below the corolla lobe sinuses
1—tube reflexed below the corolla lobe sinuses

Reflexion of the corolla tube means that a distinct corolla tube is folded back on itself below the sinuses of the corolla lobes at the level of the stamens and nectaries. This unusual condition is diagnostic for the two African endemic genera, *Basionema* and *Batesanthus*, but otherwise unknown in Periplocoideae.

11. Corolla, inside vesture
0—glabrous
1—hairy
2—papillate

12. Petal adaxial base
0—without a coronal swelling or shute
1—with a coronal swelling
2—with a coronal shute

A coronal swelling is a thickened region of coronal tissue on the surface of the corolla lobe at its base. A coronal shute is a canal formed of coronal tissue on the corolla lobe at its base. Both types form part of the primary corolline corona.

Corona

13. Corona position
0—borne just above level of nectaries
1—borne toward middle of corolla tube
2—borne at mouth of corolla tube
3—borne in sinuses of petals

14. Corona exposure
0—concealed in corolla tube
1—exserted from corolla tube mouth
2—exposed from rotate or reflexed corolla

15. Corona lobe segmentation
0—lobes unsegmented at their bases
1—lobes segmented at their bases

16. Corona annular fusion
0—lobes free from one another
1—lobes fused with one another into an annulus outside stamens, lobes alternating with corolla lobes
2—lobes fused with one another into an annulus outside stamens, lobes opposite the corolla lobes

17. Corona/staminal fusion
0—corona lobes free from stamens
1—corona lobes fused to base of stamens, without forming oblong, hemi-globose or deltoid staminal feet
2—corona lobes fused to base of stamens into oblong, hemi-globose or deltoid staminal feet, with lobes present at apex or lateral side of the staminal feet
3—corona lobes fused to base of stamens into oblong, hemi-globose or deltoid staminal feet, without any lobes present on staminal feet

Corona structure is complex in Periplocoideae. Although in some genera the corona lobes are not closely associated with the stamens, in most the free corona lobe arises from an enlarged, thickened bulge near the base of the staminal filament. This enlarged region was termed the "staminal foot" by Endress and Bruyns (2000), and is illustrated by them therein. Despite its name, the staminal foot is not merely an enlarged basal region of the stamen. Rather, it is formed by elements from both stamen and corona combined, and is thus a complex and very labile new structure without a counterpart in most plant families.

18. Corona lobe in alternipetalous position
0—absent
1—lobes filiform, linear, horn-shaped, awn-shaped, peg-like, or club-shaped
2—lobes ovate, ligulate, cordate, or hooded

19. Corona lobe apex
0—acute
1—round
2—clavate
3—emarginate
4—bifid
5—trifid to pentafid

20. Swelling between corona lobe foot and staminal foot
0—absent
1—present

In *Cryptolepis* and *Cryptostegia* the corona lobes (at middle of corolla tube) are distanced from the stamens (at base of corolla tube). The region on the inner surface of the corolla tube, between a corona lobe and the stamen directly below it, is marked by narrowly elliptic swellings.

Stamens

21. Stamen position
0—borne at level of or just above nectary lobes
1—borne distinctly above level of nectary lobes

22. Staminal filament
0—anthers borne on filaments
1—anthers subsessile

23. Anther attachment with style-head
0—anthers united with upper side of style-head
1—anthers united with margin of style-head
2—anthers united with underside of style-head
3—filaments united with style head

Not only whether or not anthers are united with the style-head, but also how and exactly where they are attached, is an important character in much of the Apocynaceae. Whereas in some subfamilies the manner of attachment is diagnostic for larger assemblages (e.g., tribes), in Periplocoideae this is not the case.

24. Anther connective appendage length
0—short, less than half as long as the anther
1—as long as or longer than the anther

25. Anther connective appendage size and shape
0—tiny, knob-like to triangular
1—at least as long as the anther, awn-like
2—at least as long as the anther, foliose

26. Anther sclerenchymatous guide rail
0—present
1—absent

Sclerenchymatous guide rails are morphologically and histologically specialized regions that develop laterally on the anthers, and they play an important role in the complex pollination mechanism of the Apocynaceae. The massive and rigid guide rails of adjacent anthers function like a track to guide the pollinator's leg or mouth parts into the narrow entrance to the pollination chamber that lies beyond. Sclerenchymatous guide rails are characteristic for Apocynoideae, Secamonoideae, and Asclepiadoideae, but are absent from Periplocoideae. The very different, more open, pollination mechanism in Periplocoideae requires that the pollinator have easy access to the nectar-

ies, in which massive sclerenchymatous guide rails blocking the entrance to the nectar would not be compatible.

27. Anther thecae vesture
    0—glabrous
    1—hairy
28. Anther connective appendage vesture
    0—glabrous
    1—hairy
29. Pollen type
    0—single-grained
    1—tetrads with inner walls double and non-perforate
    2—tetrads with inner walls thinner and perforate
    3—loosely agglutinated elongate pollinia
    4—loosely agglutinated rounded pollinia
30. Pollen pores
    0—single pollen grains 3–6-porate
    1—single pollen grains 8–16-porate
31. Nectary type
    0—nectaries arising from floral base, as a ring of lobes encircling the ovary
    1—nectaries arising from corolla tube as lobes or shelves alternating with the stamens

The nectaries found in Periplocoideae are always associated with the corolla tube or the staminal feet, whereas nectaries in Apocynoideae, when present, are associated with the ovary, often being fused to it at the base.

32. Nectary fusion
    0—free
    1—fused laterally to staminal feet forming a staminal/nectary annulus
    2—fused laterally to staminal feet and filaments, and apically also to style, forming a solid staminal/nectary tube around style
    3—fused laterally, but incompletely, with one another, and at their bases also to staminal feet and apically to style, forming a nectary tube with 5 slits around style
33. Nectary pockets
    0—absent
    1—corolline at base of corolla tube and alternating with stamens
    2—alternating with the stamens on a tube of fused staminal feet and nectaries, encircling the style

In those genera with a distinct corolla tube, e.g., *Raphionacme*, the 5 nectary lobes are altered into 5 shelves or transverse ridges near the bottom of the corolla tube. Directly above each shelf a shallow or deeper indentation is found in the corolla tube, which we call the nectary pocket. In *Hemidesmus*, unique nectary pockets are created between the staminal feet and the style through fusion of staminal feet, staminal filaments, nectaries, and style into a tube with 5 indentations or nectary pockets. Nectar collects in these pockets from the nectary shelves or nectary tube.

34. Nectary shape
    0—lobular
    1—shelf-like
    2—tubular around style and without slits
    3—tubular around style and with slits
35. Pollen translator
    0—absent
    1—present, without stipe
    2—present, with stipe

The translator is one of the key innovations in the higher Apocynaceae. The translator is a device for the transport of pollen en masse. Translators are formed around the periphery of the style-head in five grooves lined with secretory cells alternating with the adnate stamens. The translators are not cellular, but are formed from the viscous secretions of the style-head, and only become firm with desiccation. The translator of Periplocoideae is typically differentiated into three regions: an upper receptacle upon which pollen is shed, a stalk (stipe) in the middle, and a sticky disc (viscidium) at the base, which sticks the translator onto the pollinator. In most Apocynoideae style-head secretions remain viscid and do not harden into translators. In *Apocynum*, however, the secretions harden into a tacky band in the space between adjacent stamens, which have been called translators (Nilsson et al., 1993; Endress et al., 1996). We do not consider these undifferentiated bands to be homologous to the complex, differentiated translators found in Periplocoideae, however. Therefore, translators are coded as absent from *Apocynum* here.

36. Pollen translator receptacle shape
    0—flat and narrowly elliptic, narrowly ovate or oblong, not or very little folded
    1—flat and broadly ovate, broadly obovate and folded, usually horn-like
    2—hollow and spoon-like
    3—hollow and cup-like
37. Gynostegium position
    0—borne just above nectary level
    1—borne high up in corolla tube

A gynostegium is formed by a union of the androecium and the gynoecium that takes place during development. In Apocynaceae the gynostegium is formed through the union of the anthers with the specialized gynoecium apex (style-head).

38. Gynostegium exposure
    0—concealed in corolla tube
    1—exposed just above base of rotate or reflexed corolla
    2—exposed and elevated high above rotate or reflexed corolla on stilt-like filaments and style
    3—exserted from corolla tube mouth
39. Follicle structure
    0—cylindrical and not constricted
    1—cylindrical and constricted
    2—broadly keel-shaped

Appendix 2.

Generic Conspectus of Periplocoideae, Drafted from Appendix 3 for Taxonomic Novelties

Atherandra Decne., in DC., Prodr. 8: 497. 1844. TYPE: *Atherandra acuminata* Decne. 2 species.

Baroniella Costantin & Gallaud, Ann. Sci. Nat. Bot., ser. 9, 6: 354. 1908. TYPE: *Baroniella camptocarpoides* Costantin & Gallaud. 7 species.

Baseonema Schltr. & Rendle, J. Bot. 34: 97. 1896. TYPE: *Baseonema gregori* Schltr. & Rendle. 1 species.

Batesanthus N. E. Br., Hooker's Icon. Pl.: t. 2500. 1886. TYPE: *Batesanthus purpureus* N. E. Br. 4 species.

Buckollia Venter & R. L. Verh., S. African J. Bot. 60: 97. 1994. TYPE: *Buckollia volubilis* (Schltr.) Venter & R. L. Verh. Schltr. 2 species.

* Represents a taxonomic novelty herein.

Camptocarpus Decne., in DC., Prodr. 8: 493. 1844. TYPE: *Camptocarpus mauritianus* (Lam.) Decne. 9 species.

Cryptolepis R. Br., Asclepiadeae: 69. 1810. TYPE: *Cryptolepis buchananii* Roem. & Schult. 27 species.

Mangenotia Pichon*

Cryptostegia R. Br., Bot. Reg. 5: 742, t. 435. 1819. TYPE: *Cryptostegia grandiflora* Roxb. ex R. Br. 2 species, 3 varieties.

Decalepis Wight & Arn., in Wight, Contr. Bot. India: 64. 1834. TYPE: *Decalepis hamiltonii* Wight & Arn. 4 species.

Utleria Bedd. ex Benth. & Hook. f.*

Ectadium E. Mey., Comm. Pl. Afr. Austr.: 188. 1838. TYPE: *Ectadium virgatum* E. Mey. 3 species.

Epistemma D. V. Field & J. B. Hall, Kew Bull. 37: 117. 1982. TYPE: *Epistemma assianum* D. V. Field & J. B. Hall. 3 species.

Finlaysonia Wall., Pl. Asiat. Rar. 2: 48, t. 162. 1831. TYPE: *Finlaysonia obovata* Wall. 7 species.

Atherolepis Hook. f.*

Gongylosperma King & Gamble*

Hanghomia Gagnep. & Thénint.*

Meladerma Kerr*

Stelmacrypton Baill.*

Gymnanthera R. Br., Prodr.: 464. 1810. TYPE: *Gymnanthera nitida* R. Br. 2 species.

Hemidesmus R. Br., Asclepiadaceae: 45. 1810. TYPE: *Hemidesmus indicus* (L.) Schult. 1 species, 2 subspecies.

Ischnolepis Jum. & H. Perrier, Rev. Gén. Bot. 21: 53. 1909. TYPE: *Ischnolepis tuberosa* Jum. & H. Perrier. 2 species.

Petopentia Bullock*

Maclaudia Venter & R. L. Verh., J. Linn. Soc., Bot. 115: 57. 1994. TYPE: *Maclaudia felixii* Venter & R. L. Verh. 1 species.

Mondia Skeels, U.S.D.A. Bur. Pl. Industr. Bull. 223: 45. 1911. TYPE: *Mondia whitei* (Hook. f.) Skeels. 2 species.

Myriopteron Griff., Calcutta J. Nat. Hist. 4: 385. 1844. TYPE: *Myriopteron paniculatum* Griff. 1 species.

Omphalogonus Baill., Bull. Soc. Mens. Linn. Paris 2: 812. 1890. TYPE: *Omphalogonus calophyllus* Baill. 1 species.

Pentopetia Decne., in DC., Prodr. 8: 500. 1844. TYPE: *Pentopetia androsaemifolia* Decne. 21 species.

Periploca L., Sp. Pl.: 211. 1753. TYPE: *Periploca graeca* L. 13 species.

Phyllanthera Blume, Bijdr.: 1048. 1826–1827. TYPE: *Phyllanthera bifida* Blume. 8 species.

Pentanura Blume*

Streptomanes K. Schum.*

Raphionacme Harv., London J. Bot. 1: 22. 1842. TYPE: *Raphionacme divaricata* Harv. 37 species.

Sacleuxia Baill., Hist. Pl. 10: 265. 1890. TYPE: *Sacleuxia salicina* Baill. 2 species.

Sarcorrhiza Bullock, Hooker's Icon. Pl.: t 3585. 1962. TYPE: *Sarcorrhiza epiphytica* Bullock. 1 species.

Schlechterella K. Schum., in Engler & Prantl, Nat. Pflanzenfam., Index 2–4: 462. 1899. TYPE: *Schlechterella africana* (Schltr.) K. Schum. 2 species.

Stomatostemma N. E. Br., in Thiselton-Dyer, Fl. Trop. Afr. 4: 252. 1902. TYPE: *Sarcostemma monteiroae* (Oliv.) N. E. Br. 2 species.

Streptocaulon Wight & Arn., in Wight, Contr. Bot. India: 64. 1834. TYPE: *Streptocaulon kleinii* Wight & Arn. 5 species.

Tacazzea Decne., in DC., Prodr. 8: 492. 1844. TYPE: *Tacazzea venosa* Decne. 5 species.

Zacateza Bullock*

Telectadium Baill., Bull. Mens. Soc. Linn. Paris 2: 801. 1889. TYPE: *Telectadium edule* Baill. 3 species.

Zygostelma Benth., in Bentham & Hooker f., Gen. Pl. 2: 740. 1876. TYPE: *Zygostelma benthamii* Baill. 1 species.

Appendix 3.

Nomenclatural novelties within Periplocoideae.

Cryptolepis R. Br., Asclepiadeae: 69. 1810. TYPE: *Cryptolepis buchananii* Roem. & Schult.

Mangenotia Pichon, Bull. Soc. Bot. France 101: 246. 1954. **Syn. nov.** TYPE: *Mangenotia eburnea* Pichon.

Cryptolepis eburnea (Pichon) Venter, **comb. nov.** Basionym: *Mangenotia eburnea* Pichon, Bull. Soc. Bot., France 101: 246. 1954. TYPE: Ivory Coast. Abidjan, Adiopodoumé, *G-M. Mangenot s.n., anno 1951/07/06* (holotype, P not seen); Ivory Coast. Abidjan, Forest l'Abouabou, *A. J. M. Leeuwenberg 4235* (neotype, here designated, P!; isoneotypes, BR!, WAG!). [Pichon (1954) indicated the Paris Herbarium (P) as depository for the holotype specimen of *M. eburnea*, but the present first author could not find it there. As no isotypes were found elsewhere, and as it is unclear whether the sketches accompanying the type description were drawn from the holotype, a neotype is declared here for this taxon.]

The flowers of *Cryptolepis* and *Mangenotia* are similar in structure with short clavate corona lobes arising some distance above the stamens, and with characteristic swellings between the corona lobes and stamens. However, in *Cryptolepis* the corolla tube is shorter than the corolla lobes and the stamens are usually glabrous, while in *Mangenotia* the corolla tube is longer than the corolla lobes and the stamens are hairy. These differences do not merit generic distinction in the Periplocoideae.

Decalepis Wight & Arn., Contr. Bot. India: 64. 1834. TYPE: *Decalepis hamiltonii* Wight & Arn.

Utleria Bedd. ex Benth. & Hook. f., Gen. Pl. 2: 743. 1876. **Syn. nov.** TYPE: *Utleria salicifolia* Bedd. ex Benth. & Hook. f.

Decalepis salicifolia (Bedd. ex Benth. & Hook. f.) Venter, **comb. nov.** Basionym: *Utleria salicifolia* Bedd. ex Benth. & Hook. f., Gen. Pl. 2: 743. 1876. TYPE: India. Madras, *R. H. Beddome 53* (holotype, K!).

Ovate-like corona lobes, an obconical style-head with flat apex containing translator hollows, and pollen in pollinia are characteristic for *Decalepis*. *Utleria* displays similar floral characteristics.

Finlaysonia Wall., Pl. Asiat. Rar. 2: 48, t. 162. 1831. TYPE: *Finlaysonia obovata* Wall.

Atherolepis Hook. f., Hooker's Icon. Pl.: t. 433. Dec. 1883. **Syn. nov.** TYPE: *Atherolepis wallichii* (Wight) Hook. f.

Gongylosperma King & Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74: 510. 1908. **Syn. nov.** TYPE: *Gongylosperma curtisii* King & Gamble.

Hanghomia Gagnep. & Thénint. Bull. Soc. Bot. France 83: 392. 1936. **Syn. nov.** TYPE: *Hanghomia marseillii* Gagnep. & Thénint.

Meladerma Kerr, Kew Bull. 1938: 445. 1938. **Syn. nov.** TYPE: *Meladerma puberulum* Kerr.

Stelmacrypton Baill., Bull. Mens. Soc. Linn. Paris 2: 812. 1889. **Syn. nov.** TYPE: *Stelmacrypton khasianum* (Kurz) Baill.

Finlaysonia wallichii (Wight) Venter, **comb. nov.** Basionym: *Hemidesmus wallichii* Wight, Contr. Bot. India: 63. 1834. *Atherolepis wallichii* (Wight) Hook. f., Fl. Brit. India 4(10): 8. June 1883. TYPE: [Burma], in collibus Prome, *N. Wallich 8245* (*asclep. 106*) (holotype, K-W!; isotype, K!). [In the protologue of *Hemidesmus wallichii* the type is indicated as "*Wallich asclep. 106*," but it is not to be found at K, or elsewhere. Fortunately, in the Wallich Herbarium (K-W), under the above species name, there is a specimen *8245* with "*asclep. 106*" written on the sheet. In the general collection at K, specimen *8245* is considered to be the type of *Atherolepis wallichii* [= *Hemidesmus wallichii* Wight], and we follow this indication as correct.]

Finlaysonia pierrei (Costantin) Venter, **comb. nov.** Basionym: *Atherolepis pierrei* Costantin, in Lecomte, Fl. Gen. Indo-Chine 4: 29. 1912. TYPE: Thailand. Prov. Bien-hoa, Bao-chiang, *Pierre 4376* (lectotype, here designated, P!; isolectotypes, BM!, P 6 sheets!, K 2 sheets!).

Finlaysonia curtisii (King & Gamble) Venter, **comb. nov.** Basionym: *Gongylosperma curtisii* King & Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74: 510. TYPE: Thailand. Pang-nga, Poongah, *C. Curtis 2591* (holotype, CAL?; isotype, K!). [George King was superintendent of the Calcutta Botanic Garden in India, and James Sykes Gamble was a British forester and botanist in India (Stafleu & Cowan, 1976, 1979). As the type specimen at the Kew Herbarium (K) is not indicated as the holotype, we assume that the holotype is at the Calcutta Herbarium (CAL) but have not seen it. In light of this uncertainty we refrain from designating the Kew specimen as lectotype of the species.]

Finlaysonia lanuginosa (Ridley) Venter, **comb. nov.** Basionym: *Gongylosperma lanuginosa* Ridley, J. Asiat. Soc. Straits 59: 135. 1911. TYPE: Malaya. Lankawi Island, near Dayong Bonting, C. Curtis 2663 (holotype, SING?; isotype, K!?). [The same reasoning as above is true for the holotype of this species, as Henry Nicholas Ridley was Director of the Botanic Gardens of Singapore when he published *G. lanuginosa* (Stafleu & Cowan, 1983). The situation is further complicated by the fact that the specimen at K, indicated as type of the species, is without any collector's number, but from the same locality.]

Finlaysonia insularum (King & Gamble) Venter, **comb. nov.** Basionym: *Gymnanthera insularum* King & Gamble, J. Proc. Asiat. Soc. Bengal 74: 514. 1907. *Meladerma insularum* (King & Gamble) Kerr, Kew Bull. 1938: 445. 1938. TYPE: Malaya, Kedah, small islands near Langkawi, *C. Curtis 3684* (holotype, CAL?; isotype, K!). [See discussion about the Calcutta Herbarium under *Finlaysonia curtisii*.]

Meladerma puberulum Kerr, Kew Bull. 1938: 447. 1938. **Syn. nov.** TYPE: Thailand, Prachuap, *Put 285* (holotype, K!; isotype, L!).

Meladerma deciduum Kerr, Kew Bull. 1938: 447. 1938. **Syn. nov.** TYPE: Thailand. Chiengmai, Ban Pa Sak, *Kerr 2834* (lectotype, here designated, K sheet 1!; isolectotypes, BM!, K sheet 2!). [Not one of the two type specimens at K was designated as holotype; a lectotype is thus called for.]

Finlaysonia khasiana (Kurz) Venter, **comb. nov.** Basionym: *Pentanura khasiana* Kurz, in Hook. f., Fl. Brit. India 4: 4. 1885. *Stelmacrypton khasiana* (Kurz) Baill., Bull. Mens. Soc. Linn. Paris 2: 812. 1889. TYPE: India. Khasia Mountains, *Kurz*, commissioned from Dr. King, anno 03/1883 (lectotype, here designated, K!); Khasia Mountains, 3–4000 ft., commissioned from HBC, anno 08/78 (syntype, K!); Khasia Mountains, *J. D. Hooker & T. Thomson*, anno 1859 (syntypes, BM!, P!). [The type data provided with the protologue of *Pentanura khasiana* is not very clear, namely: "Khasia Mts. alt. 3–4000 ft., J. D. H. & T. T., Kurz." The collections indicated are confusing. Two sheets are present in the type file of *Pentanura khasiana* in the Kew Herbarium (K). Kurz certainly did not collect them, neither did J. D. Hooker, but the two sheets were commissioned from the collectors Dr. King and HBC. We have little doubt that the sheet commissioned from Dr. King is one of the syntypes, and the "3–4000 ft." written on the second sheet, commissioned from HBC, indicates that this is another syntype sheet. However, there is no specimen of J. D. H. & T. T. in the type file of the Kew Collection, but such a specimen, collected by J .D. Hooker & T. Thomsom, exists and is filed under *Stelmacrypton khasiana* in the British Museum Herbarium (BM), also in the Paris Herbarium (P), and this must be the missing syntype.

Hanghomia marseillii Gagnep. & Thénint., Bull. Soc. Bot. France 83: 392. 1936. **Syn. nov.** TYPE: Laos. Region Xieng-Khonang, Plateau Tran-Ninh, *M. André s.n.* (holotype, P!).

Periploca acuminata Rahman & Wilkock, J. Linn. Soc., Bot. 110: 373. 1992. **Syn nov.** TYPE: Bangladesh. Sylhet Tamabil-Jaflong Hills, *Rahman & Hossain 56* (holotype, ABD!; isotypes, BM!, K 2 sheets!)

Finlaysonia, *Atherolepis*, *Gongylosperma*, and *Meladerma* have in common an exposed gynostegium from a rotate or saucer-shaped corolla, their staminal feet swollen and these topped by corona lobes, and their pollen fused into pollinia instead of into tetrads.

Ischnolepis Jum. & H. Perrier, Rev. Gén. Bot. 21: 53. 1909. TYPE: *Ischnolepis tuberosa* Jum. & H. Perrier.

Petopentia Bullock, Kew Bull. 1954: 32. 1954. **Syn. nov.** TYPE: *Petopentia natalensis* (Schltr.) Bullock.

Ischnolepis natalensis (Schltr.) Venter, **comb. nov.** Basionym: *Petopentia natalensis* Schltr., J. Bot. Lond. 32: 257. 1894. *Tacazzea natalensis* (Schltr.) N. E. Br., Fl. Cap. 4: 514. 1907. *Petopentia natalensis* (Schltr.) Bullock, Kew Bull. 1954: 362. 1954. TYPE: South Africa. Kwazulu-Natal, Isipingo, *Wood s.n.* (holotype, K!).

Although separated widely, *Ischnolepis* in Madagascar and *Petopentia* in South Africa, there exists a conspicuous and characteristic similarity in the flowers of these two monotypic genera. Their corollas are rotate and greenish yellow, having narrowly triangular corolla lobes with acute apices and rather thick texture. Vegetatively both genera

possess subterranean tubers and a peculiar wax-like, maroonish stem bark.

Phyllanthera Blume, Bijdr.: 1048. 1826–1827. TYPE: *Phyllanthera bifida* Blume.

Pentanura Blume, Mus. Bot. 1: 125. 1850. **Syn. nov.** TYPE: *Pentanura sumatrana* Blume.

Streptomanes K. Schum., in Schumann & Lauterbach, Nachträge. Fl. Schutzgeb. Südsee: 352. 1905. **Syn. nov.** TYPE: *Streptomanes nymanii* K. Schum. [Incorrectly synonymized with *Cryptolepis* by Forster (1990).]

Phyllanthera grayi (F. I. Forst.) Venter, **comb. nov.** Basionym: *Cryptolepis grayi* F. I. Forst., Austrobaileya 3: 279. 1990. TYPE: Australia. Tolga Scrub, *B. Gray 1561, 1979/11/09* (holotype, QRS!; isotype, BRI!).

Phyllanthera lancifolia (P. I. Forst.) Venter, **comb. nov.** Basionym: *Cryptolepis lancifolia* P. I. Forst., Blumea 35: 381. 1991. TYPE: New Guinea. Irian Jaya, Okwalimkan River headwaters, *Ridsdale, Henty & Galore, NGF-31999, 1967/08/17* (holotype, L!; isotype, LAE not seen).

Phyllanthera multinervosa (F. I. Forst.) Venter, **comb. nov.** Basionym: *Cryptolepis multinervosa* F. I. Forst., Austrobaileya 4: 70. 1993. TYPE: Papua New Guinea. Southern Highlands Province, near Waro Airstrip, *M. Jacobs 9287, 1973/10/14* (holotype, L!; isotypes, L!, CANB & LAE not seen).

Phyllanthera papillata (F. I. Forst.) Venter, **comb. nov.** Basionym: *Cryptolepis papillata* F. I. Forst., Austrobaileya 3: 277. 1990. TYPE: Papua New Guinea. Morobe District. Bulolo Valley, Partep Creek, Laewau road, *J. S. Womersley NGF-7821, 1995/09/00* (holotype, BRI!; isotypes, L!, BO & K not seen).

Phyllanthera sumatrana (Blume) Venter, **comb. nov.** Basionym: *Pentanura sumatrana* Blume, Mus. Bot. 1: 125. 1850. TYPE: *Herb. Lugd. Bat. 898, 169-54* (holotype, L!).

Phyllanthera nymanii (K. Schum.) Venter, **comb. nov.** Basionym: *Streptomanes nymanii* K. Schum., in Schumann & Lauterbach, Nachträge Fl. Schutzgeb. Südsee: 352. 1905. *Cryptolepis nymanii* (K. Schum.) F. I. Forst., Austrobaileya 3: 275. 1990. TYPE: Kaiser-Wilhemsland (Papua New Guinea), Stephansort, *E. O. A. Nyman 1020* (holotype, B destroyed; lectotype, here designated, UPS, isotypification by Forster, 1990).

Phyllanthera, Pentanura, and Streptomanes display similar flowers with rotate corollas, swollen staminal feet lacking any corona lobes, nectaries fused into a conical nectary tube around and with the style, prominent anther apices, and pollen in tetrads. The four species described under *Cryptolepis* also belong to *Phyllanthera* as they have the same peculiar floral structure.

Streptocaulon Wight & Arn., Contr. Bot. India: 61. 1834. TYPE: *Streptocaulon kleinii* Wight & Arn., lectotype here designated. [Wight, when he described *Streptocaulon*, did not indicate a generitype, but he added descriptions of six new *Streptocaulon* species. The first species listed was *Streptocaulon tomentosum* Wight, but as this is a synonym of *Streptocaulon juventas* (Lour.) Merr., we designate the second listed species, *Streptocaulon kleinii*, as the generitype.]

Streptocaulon cumingii (Turcz.) Vill., in Blanco, Fl. Filip., ed. 3, Nov. App.: 132. 1877. *Tripolepis cumingii* Turcz, Bull. Soc. Imp. Naturalistes Moscou 21: 251. 1848. TYPE: Phillipines, Prov. Pangasanan, Luzon (Lucon), *Cuming 1025* (MW?); Albay, Luzon, *Cuming 1024* (BM!, K×2!, P×2!, W×3!). [Turczaninow indicated only *Cuming 1025* as type with his protologue of *Tripolepis cumingii*. However, *Cuming 1024* is regarded as type of the species in all the herbaria indicated above. As we were unable to obtain the type from the Moscow Herbarium, we, at present, indicate no holo-, lecto- or neolectotype specimen for *S. cumingii*].

Streptocaulon corymbosum (Elmer) Elmer, Leafl. Philipp. Bot. 10: 3594. 1938. **Syn. nov.** *Anodendron corymbosum* Elmer, Leafl. Philipp. Bot. 2: 512. 1908. TYPE: Philippines. Luzon, *Elmer 8468* (holotype, PNH?; isotypes, G!, K!). [Many of Adolph Daniel Edward Elmer's types are housed in the Philippine National Herbarium (PNH) in Manila (Stafleu & Cowan, 1976), and as he described his new species in a Philippine journal one can expect the holotype to be at PNH. However, as yet we have had no opportunity to search for it at PNH.]

Streptocaulon wallichii Wight, Contr. Bot. India: 65. 1834. TYPE: Malaya. Penang & Singapore, *Wallich, asclep. n. 124* (lectotype, here designated, K!; isolectotype, K!).

Streptocaulon baumii Decne., in DC., Prodr. 8: 496. 1844. **Syn. nov.** TYPE: Philippines. Manilla, *Baum & Perrottet* (holotype, P not seen); *Baum, anno 1832/02/08* (neotype, here designated, P!). [The specimen *Baum, anno 1832/02/08*, may indeed be the holotype, but as uncertainty exists, we rather declare it a neotype for the species.]

Tacazzea Decne., in DC., Prodr. 8: 492. 1844. TYPE: *Tacazzea venosa* Decne.

Zacateza Bullock, Kew Bull. 1954: 361. 1954. **Syn. nov.** TYPE: *Zacateza pedicellata* (K. Schum.) Bullock [basionym: *Tacazzea pedicellata* K. Schum., in Engler, Bot. Jahrb. 17: 115. 1893]. TYPES: Democratic Republic of the Congo. Munsa, Monbuttu-land, *Schweinfurth 3483* (holotype, B destroyed; lectotype, here designated, K!); *Schweinfurth 3488* (syntype, K!).

Bullock (1954) separated *Tacazzea pedicellata* from *Tacazzea* because of its long-pedicelled, smaller inflorescences and its elliptic leaves with patent venation. However, the flowers of *Tacazzea* and *Zacateza* are similar. We do not regard Bullock's distinctions as valid reasons for separating this species as a genus from *Tacazzea*.

Appendix 4.

Key to the Genera of the Periplocoideae

1. Gynostegium enclosed and concealed within the corolla tube 2
1′. Gynostegium exposed from short-tubed corolla (corolla usually rotate), or gynostegium in mouth of, or exserted from, corolla with distinct tube 9
2(1). Flowers large, 3–6 cm long and 5–9 cm diam. (Madagascar) ..... *Cryptostegia*
2′. Flowers small, less than 2 cm long and less than 3 cm diam 3

3(2). Corolla sub-hemispherical to urceolate ... 4
3′. Corolla campanulate to cylindrical ... 5
4(3). Corolla sub-hemispherical to urceolate with 5 deep vertical cavities (eastern Asia) ... *Telectadium*
4′. Corolla urceolate, without any cavities (Madagascar) ... *Pentopetia*
5(3). Corona in mouth of corolla tube or in sinuses of petals ... 6
5′. Corona arises lower down in corolla tube, mostly around the middle of the tube ... 8
6(5). Corona in sinuses of the petals; lobes club- or matchstick-like (southern Africa) ... *Stomatostemma*
6′. Corona in corolla mouth, just below petal sinuses; lobes acicular or corniculate ... 7
7(6). An erect shrub with pale green to yellow, 8–12 mm long, trumpet-shaped corolla (southwestern Africa) ... *Ectadium*
7′. A climber with very small, dark red, 2–3 mm long, campanulate corolla (West Tropical Africa) ... *Maclaudia*
8(5). Inside of corolla tube and adaxial side of petals papillate; corona of vertical ridges, with hooded apices, radiating into the corolla tube cavity, without a spongy swelling between corona lobe base and stamen base (tropical Africa) ... *Omphalogonus*
8′. Inside of corolla tube and adaxial side of petals glabrous; corona lobes clavate, filiform or acicular, almost always with a spongy pad between corona lobe base and stamen base (Africa, Arabia, and Eastern Asia) ... *Cryptolepis*
9(1). Pollen in pollinia ... 10
9′. Pollen in tetrads ... 16
10(9). Corona lobes fused into an annulus outside the stamens (eastern Africa) ... *Schlechterella*
10′. Corona lobes not fused into an annulus outside the stamens ... 11
11(10). Corolla tube conspicuous and cylindric with stamens and corona lobes arising from the corolla tube mouth (eastern Asia to Australia) ... *Gymnanthera*
11′. Corolla tube bowl-shaped to indistinct and rotate ... 12
12(11). Inflorescence a multi-flowered cymose panicle (eastern Asia) ... *Streptocaulon*
12′. Inflorescence a 1–10-flowered cyme ... 13
13(12). Petals valvate in bud; interstaminal nectary lobes fused into a tube around the style, creating 5 pockets between the style and stamens (southern Asia) ... *Hemidesmus*
13′. Petals contorted in bud; interstaminal nectaries not fused around the style, not forming any pockets between the style and stamens ... 14
14(13). Style-head with a flat or concave apex on which 5 hemispherical hollows include the receptacles of the pollen translators (southern Asia) ... *Decalepis*
14′. Style-head apex deltoid or rounded, without any hemispherical translator hollows ... 15
15(14). An epiphyte; corolla tube with 5 radial ridges; corona lobes with dilated hairy apex (tropical Africa) ... *Epistemma*
15′. A climber with roots in the ground; corolla without radial ridges; corona lobes not dilated at apex, not hairy (Asia to Australia) ... *Finlaysonia*
16(9). An epiphytic plant with aerial tubers (tropical Africa) ... *Sarcorrhiza*
16′. Climbers, shrubs, or herbs anchored in the ground, if tubers present then subterranean or on ground surface ... 17
17(16). Corona lobes absent, at least opposite the sepals ... 18
17′. Corona lobes present opposite the sepals ... 22
18(17). Anthers with their connective apices conspicuously enlarged and hood-shaped, ovate, or strap-shaped; nectary lobes fused together in form of a conical tube with slits; tube fused to the style at its apex (Southeast Asia) ... *Phyllanthera*
18′. Anther connective apices not enlarged as above; nectary lobes free from one another and free from the style ... 19
19(18). An undulating coronal annulus present outside the staminal filaments, and with corona lobes opposite the petals (Madagascar) ... *Baroniella*
19′. Bases of staminal filaments and corona lobes fused into staminal feet, these staminal feet fused together into an annulus outside the nectary lobes, or the staminal feet and nectary lobes fused together into an annulus ... 20
20(19). Erect shrubs; flowers very small, 2–3 mm long and less than 5 mm diam. (eastern Africa) ... *Sacleuxia*
20′. Climbers; flowers larger, from 5–20 mm long and 8–30 mm diam ... 21
21(20). Staminal feet fused laterally with one another into a circular coronal annulus, nectary lobes fused to inner base of coronal annulus; stem bark maroonish grayish, glossy, flaky, wax-like and glabrous (tropical Africa) ... *Batesanthus*
21′. Staminal feet and nectary lobes fused into an undulating coronal annulus; stem bark brown and hairy (eastern Africa) ... *Baseonema*
22(17). Corona lobes fused into an annulus outside the stamens, or staminal feet fused laterally into a coronal annulus on a reflexed corolla tube ... 23
22′. Corona lobes not fused into an annulus outside the stamens; staminal feet not fused into a coronal annulus on a reflexed corolla tube ... 25
23(22). Corolla tube reflexed; staminal feet fused laterally into an annulus; stem bark maroonish grayish, glossy, flaky and wax-like (tropical Africa) ... *Batesanthus*
23′. Corolla tube not reflexed, corona lobes fused into an annulus outside the stamens; stem bark not as above ... 24
24(23). Erect suffrutescent herbs (tropical Africa) ... *Raphionacme*
24′. Woody suffrutescent climbers (Madagascar and the Mascarene Islands) ... *Camptocarpus*
25(22). Inflorescences multi-flowered cymose open panicles, or dense spike-like to globose panicles ... 26
25′. Inflorescences with 1–10-flowered cymes ... 30
26(25). Interpetiolar collar with green frills or reddish colleters present ... 27
26′. Only interpetiolar lines may be present, reddish colleters may also be present ... 28
27(26). Interpetiolar collar with green frills; flowers large, 15–25 mm diam., follicles smooth (tropical and subtropical Africa) ... *Mondia*
27′. Interpetiolar collar with reddish colleters;

flowers small, up to 10 mm diam., follicles winged (eastern Asia) ..... *Myriopteron*

28(26). Erect or climbing herbaceous tuberous geophytes; flowers in globose or spike-like panicles (tropical and southern Africa) ..... *Raphionacme*

28′. Perennial climbers or erect shrubs; inflorescences of multi-flowered open panicles 29

29(28). Stamens hairy (Africa, Arabia, Europe, and Asia) ..... *Periploca*

29′. Stamens glabrous (tropical and subtropical Africa) ..... *Tacazzea*

30(25). Corona lobes arise in petal sinuses (Madagascar) ..... *Pentopetia*

30′. Corona lobes arise from the corolla tube 31

31(30). Apical appendages of anther connectives combined into a long lance-like cone above the style-head (Asia) ..... *Atherandra*

31′. Apical appendages of anther connectives short and connivent into a round or acute cover above style-head 32

32(31). Erect or climbing herbaceous geophytes, rarely woody perennial climbers; corolla tube campanulate to cylindrical with the stamens and corona lobes arising from the tube mouth (Africa and Arabia) ..... *Raphionacme*

32′. Woody perennial climbers or erect shrubs; corolla rotate with tube nearly indistinct 33

33(32). Stamens hairy (Africa, Arabia, Europe, and Asia) ..... *Periploca*

33′. Stamens glabrous 34

34(33). Petals yellow inside, narrowly triangular with sharp apex (southern Africa and Madagascar) ..... *Ischnolepis*

34′. Petals pink, maroon, or pink-whitish inside, apices round 35

35(34). An erect, tuberous shrub with small flowers 2–3 mm long; ovary may be hairy (eastern Africa) ..... *Sacleuxia*

35′. Climbers with flowers 5–10 mm long; ovaries glabrous 36

36(35). Corona lobes filiform with undivided apex (eastern Africa) ..... *Buckollia*

36′. Corona trifid, middle segment acicular, erect and shorter than the curved lateral segments (eastern Asia) ..... *Zygostelma*

POLLEN MORPHOLOGY OF THE PERIPLOCOIDEAE, SECAMONOIDEAE, AND ASCLEPIADOIDEAE (APOCYNACEAE)[1]

Rudolf L. Verhoeven[2] *and*
H. Johan T. Venter[2]

ABSTRACT

Among Apocynaceae the Periplocoideae are distinguished from the Secamonoideae and Asclepiadoideae by the presence of free tetrads or pollinia, which are shed onto spoon-shaped translators after anthesis. The Secamonoideae are distinguished from Asclepiadoideae by having four pollinia attached to a translator, rather than two pollinia as in Asclepiadoideae. In the Periplocoideae the exine consists of a tectum and a granular stratum. In Secamonoideae the exine consists of three layers: an outer tectum, subtended by a well-developed granular layer, which is followed by a foot layer. In the Asclepiadoideae the exine consists of a tectum (distal exine layer), a thin granular layer, and an inner foot layer.

Key words: Apocynaceae, Asclepiadoideae, Periplocoideae, pollen morphology, Secamonoideae.

The Periplocoideae, Secamonoideae, and Asclepiadoideae can be classified as subfamilies of the Asclepiadaceae (Liede & Albers, 1994), or they can be classified as subfamilies of an extended Apocynaceae formed by the amalgamation of Apocynaceae and Asclepiadaceae (Sennblad & Bremer, 1996; Endress & Bruyns, 2000). Three tribes have been recognized in the Periplocoideae: Periploceae, Gymnanthereae, and Cryptolepideae (Venter & Verhoeven, 1997), though more recent studies suggest that these may not be natural groups (Venter & Verhoeven, 2001 this volume). The Secamonoideae, with only nine genera (Klackenberg, in press), are not subdivided into tribes, whereas in the Asclepiadoideae five tribes are often recognized: Fockeeae, Marsdenieae, Stapelieae, Gonolobeae, and Asclepiadeae (Liede & Albers, 1994). In some recent studies the Gonolobeae are nested within the Asclepiadeae, and are thus not recognized as a separate tribe (Liede, 1997; Potgieter & Albert, 2001 this volume). Recently the families Asclepiadaceae and Apocynaceae were grouped together into one family, the Apocynaceae s.l. (Endress & Bruyns, 2000). Endress and Bruyns (2000) reduced the tribes of the Asclepiadoideae to three by abandoning Fockeeae and placing it in the tribe Marsdenieae, and abandoning Gonolobeae and placing it in the Asclepiadeae. Further, because of the priority rule, the name Ceropegieae was adopted for the tribe formerly known as Stapelieae, in compliance with *Index Nominum Supragenoricum Plantarum Vascularium* (see J. Reveal's website: <http://matrix.nal.usda.gov:8080/star/supragenericname.html>). In the present paper the tribe Fockeeae is maintained. Palynologically the Periplocoideae are distinguished from Secamonoideae and Asclepiadoideae by the presence of tetrads, or free pollinia (in 7 genera: Verhoeven & Venter, 1998a, 1998b; Venter & Verhoeven, 2001), which are shed onto translators at anthesis. The Secamonoideae are distinguished from Asclepiadoideae by having four pollinia attached to a translator, as opposed to two pollinia in Asclepiadoideae. In the Secamonoideae and Asclepiadoideae, at the last stage of pollinarium development, when the anther wall dehisces, the pollinium comes into contact with, and becomes attached to, the translator apparatus (Kunze, 1994; Omlor, 1996; Civeyrel et al., 1998). The pollinium is not released from the anther at this stage. In the Secamonoideae and Asclepiadoideae the pollinium remains in the anther locule until the pollinarium is removed by the pollinator. In the Periplocoideae the translator is attached to the pollinator by means of an adhesive disc, while in the Secamonoideae and Asclepiadoideae it is a clasping mechanism.

Pollinia of different genera and species vary in morphological respects. Galil and Zeroni (1969), El-Gazzar and Hamza (1973), El-Gazzar et al. (1974), Schill and Jäkel (1978), Dannenbaum and Schill (1991), Nilsson et al. (1993), and Civeyrel

[1] The financial support from the National Research Foundation and the University of the Free State is gratefully acknowledged. All the herbaria mentioned are thanked for the kind loan of their specimens.

[2] Department of Botany & Genetics, University of the Free State, P. O. Box 339, Bloemfontein 9300, South Africa.

Table 1. Pollinarium and pollinium characters of Periplocoideae, Secamonoideae, *Fockea*, and Asclepiadoideae (excl. *Fockea*). The definition of pollinarium is taken from Endress (1994: 472) as the "apparatus for the transport of pollinia (the pollinia included)." In Asclepiadoideae (excl. *Fockea*), the characters "exine" and "distal walls" refer to the pollinium wall.

	Periplocoideae	Secamonoideae	*Fockea*	Asclepiadoideae (excl. *Fockea*)
Manner of attachment to pollinator	Viscidium (adhesive disc)	Corpusculum	Corpusculum	Corpusculum
Number of pollinia per anther	4 (loose pollinia)	4	2	2
Pollinium wall	Absent	Absent	Absent	Present
Composition of pollinium	Tetrads	Tetrads	Tetrads	Single grains
Exine	2-layered (tectum and granular stratum)	3-layered (tectum, well-developed granular layer and foot layer)	3-layered (tectum, thin granular layer and foot layer)	3-layered (tectum, thin granular layer and foot layer)
Distal walls (exine and intine)	3-layered	4-layered	4-layered	4-layered
Proximal walls (exine and intine)	3-layered	2-layered	2-layered	Not applicable

(1994, 1995) provided an overview of pollinium and/or pollinarium structure in the family. The purpose of this study is to provide information concerning the pollinium and pollinium wall structure of the three subfamilies according to our own observations, and is not meant to be a comprehensive palynological study.

Material and Methods

Tetrads and pollinia were obtained from herbarium specimens and fresh material collected in the field. Herbaria from which material was obtained are BLFU, BM, BR, K, L, MO, NH, P, PRE, SRGH, WAG, and WIND. Specimens investigated are indicated in Appendix 1.

Material collected in the field was fixed and flowers obtained from herbarium specimens were rehydrated in 3% phosphate-buffered glutaraldehyde. For scanning electron microscopy (SEM) pollinaria were removed from flowers in 100% ethanol. The pollinaria were air dried and mounted on stubs using double-sided tape, coated with gold, and examined with a Jeol Winsem 6400 microscope at 5 kV. For transmission electron microscopy (TEM) the pollinia were postfixed in 2% osmium tetroxide, stained with 0.5% uranyl acetate, dehydrated in an alcohol series, and embedded in Spurr's low-viscosity resin. Sections were stained with uranyl acetate, followed by lead citrate, and examined with a Philips CM 100 electron microscope at 60 kV.

Pollinium morphology is as described by Verhoeven and Venter (1994a). A pollinium is the contents of one microsporangium (pollen sac), forming one pollen-unit. Endress (1994: 472) defined pollinarium as the "apparatus for the transport of pollinia (the pollinia included) as occurring in Asclepiadaceae and Orchidaceae." A massula is considered to be a pollen unit larger than a polyad, but not the entire product of one microsporangium (pollen sac). Accordingly, each anther in the Periplocoideae produces four free pollinia, not massulae, as was reported by Nilsson et al. (1993). The terminology used for the walls of the pollinium and tetrads (Verhoeven & Venter, 1998a) is as follows: The distal walls are the tetrad walls that face toward the outside (circumference) of the pollinium. The proximal walls are the walls that separate tetrads on the inside of the pollinium. The inner walls are the walls that separate individual pollen grains of a tetrad. See also Table 1 for additional details of pollinaria and pollinia characters.

Results

Periplocoideae

The Periplocoideae are characterized by having tetrads or free pollinia, which are shed onto cone-shaped or spoon-like translators, each of which consists of an adhesive disc (by which the translator sticks to the pollinator), a stalk and an adhesive-lined receptacle (onto which the tetrads or pollinium is shed at anthesis), as exemplified by *Finlaysonia* Wall. (Fig. 1A). The five translators present in a *Raphionacme* Harv. flower are positioned be-

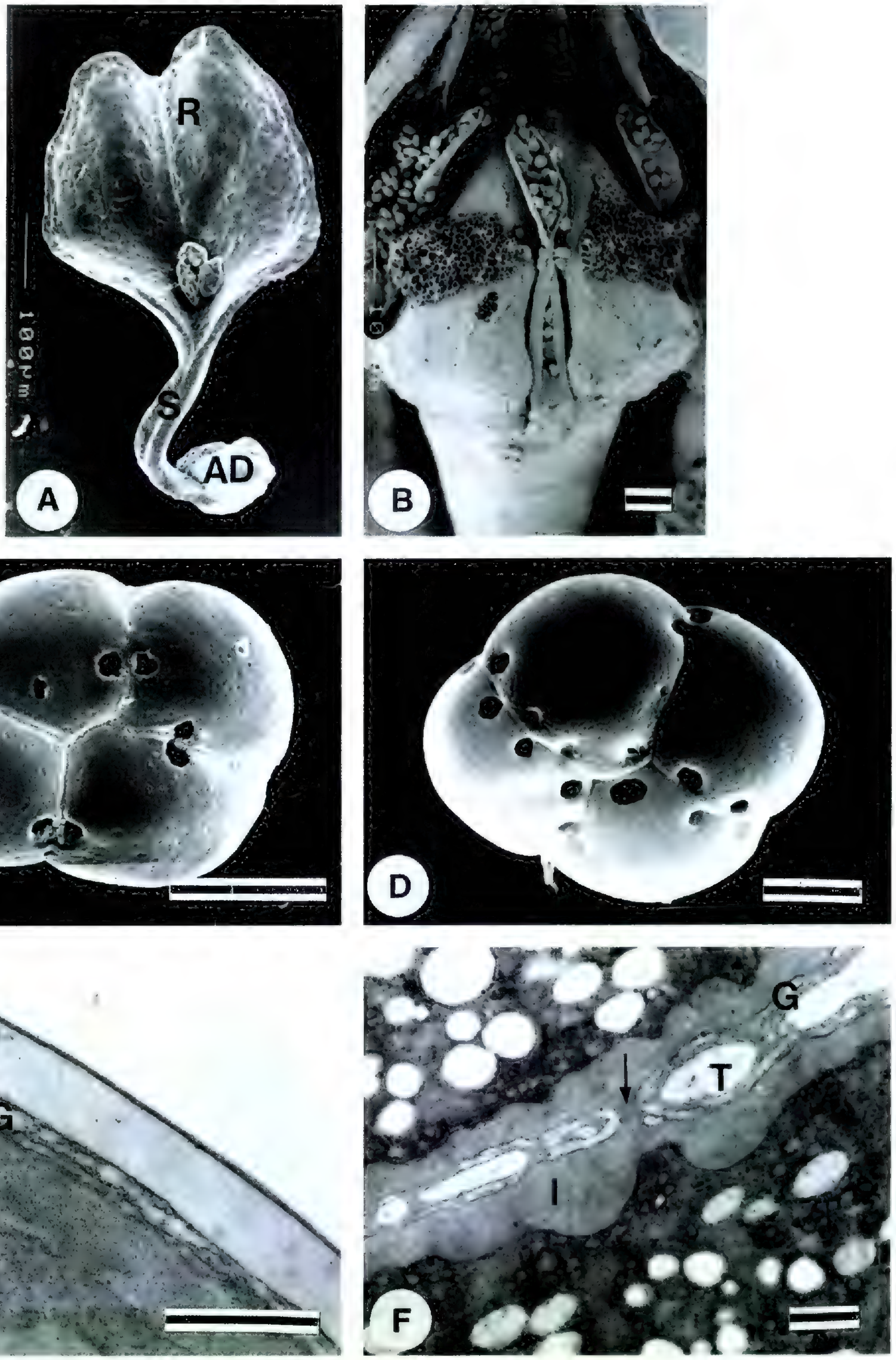
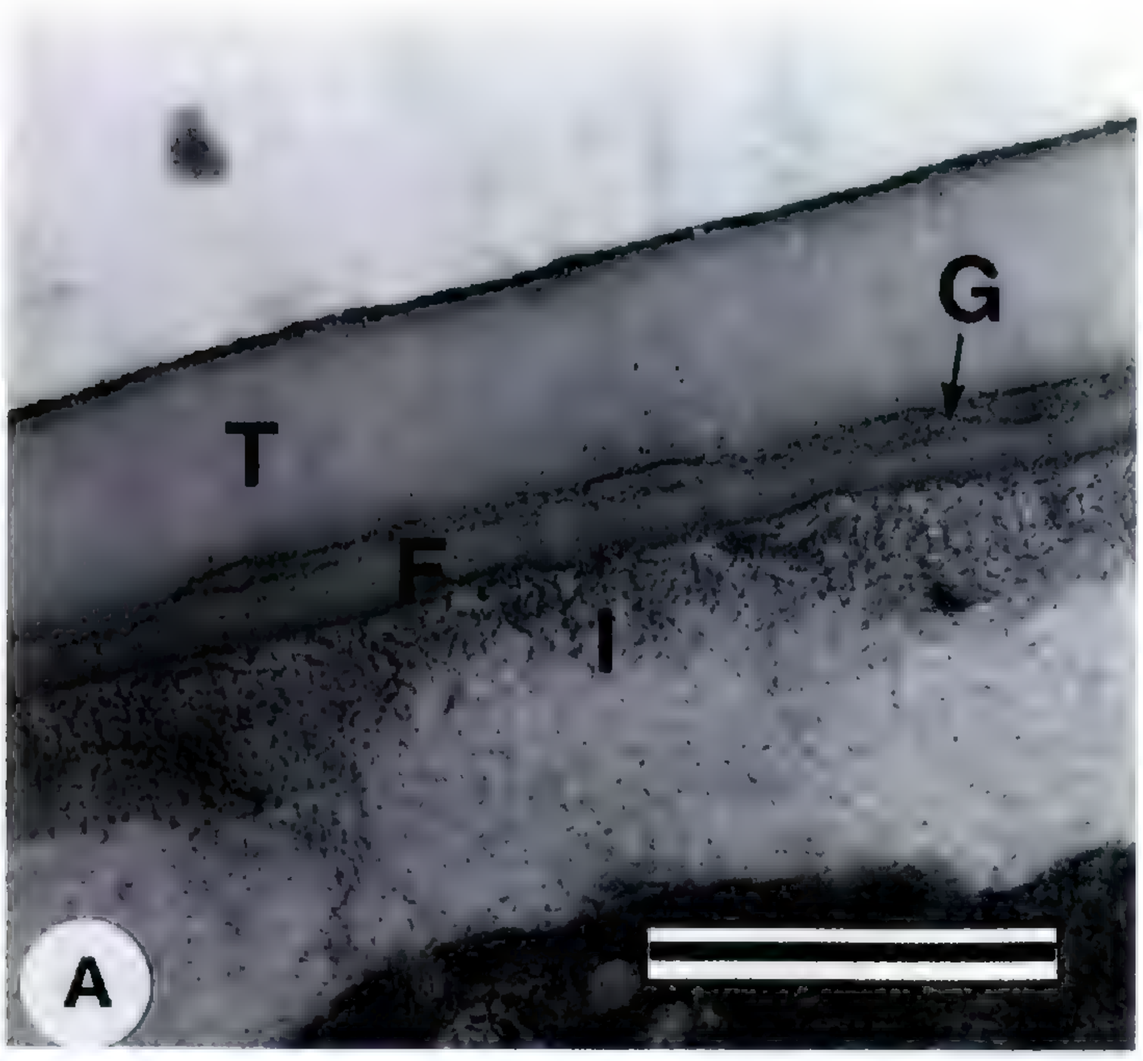
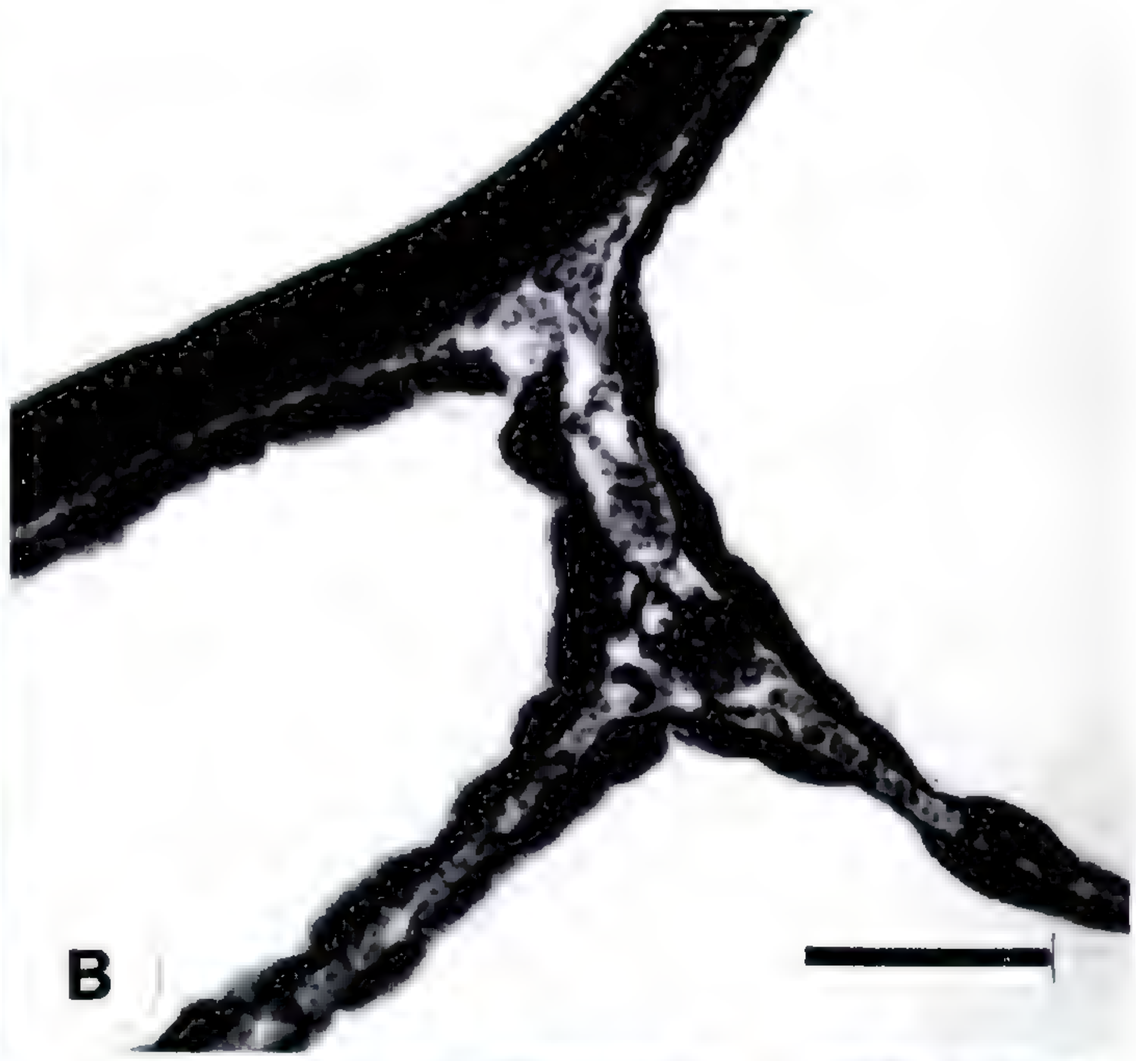


Figure 1. A. *Finlaysonia khasiana*. Translator showing adhesive disc (AD), stalk (S), and receptacle (R). —B. *Raphionacme hirsuta*. Translator positioned between anthers (removed) in a groove on stigmatic head. —C. *Cryptolepis albicans*. Rhomboidal tetrad. —D. *Raphionacme madiensis*. Rhomboidal tetrad. —E. *Raphionacme hirsuta*. Section of wall showing tectum (T), granular stratum (G), and intine (I). —F. *Raphionacme palustris*. Inner wall showing wall bridge (arrow), tectum (T), granular stratum (G), and intine (I). Scale bars: A & B = 100 μm; C = 20 μm; D = 10 μm; E & F = 1 μm.





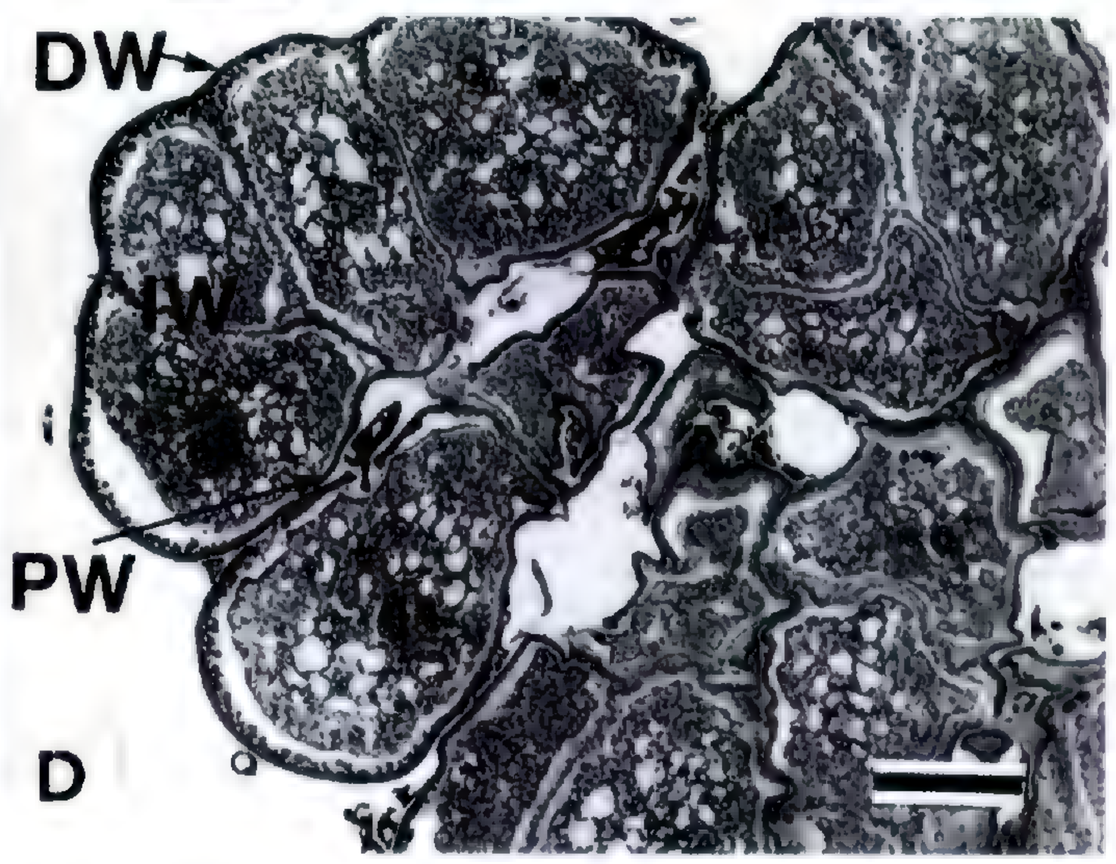


Figure 2. A, B. *Camptocarpus linearis*. —A. Section of wall showing tectum (T), granular stratum (G), foot layer (F), and intine (I). —B. Section of acetolyzed tetrad showing outer and inner wall of tetrad. C, D. *Hemidesmus indicus*. —C. Pollinium. —D. Section of pollinium showing tetrads, distal (DW), proximal (PW), and inner wall (IW). Scale bars: A = 0.5 µm; B = 1 µm; C & D = 10 µm.

tween the anthers, with the stalk fitting in a groove on the stigmatic head (Fig. 1B). Translator, tetrad, and pollinium morphology of Periplocoideae have been described by Schick (1982), Kunze (1993), Nilsson et al. (1993), Verhoeven et al. (1989), and Verhoeven and Venter (1988, 1993, 1994a, b, 1997, 1998a, b). The tetrad and pollinium morphology of the Periplocoideae will be discussed very briefly for the purpose of this paper (see also Table 1). The calymmate tetrads are generally arranged rhomboidally or decussately; however, tetragonal, tetrahedral, linear, and T-shaped arrangements are also present in some genera. The number of pores varies from 4 to 6 in most genera, such as *Cryptolepis* R. Br. (Fig. 1C). *Raphionacme*, however, has 8 to 16 pores per pollen grain (Fig. 1D). In all the genera the exine is smooth and consists of a distal stratum (tectum) subtended by a granular stratum (Fig. 1E) as seen in *R. hirsuta* (E. Mey.) R. A. Dyer. The inner walls separating the individual grains of the tetrad have the same structure as the exterior wall, consisting of tectum, granular stratum, and intine. The walls are, however, not continuous, but interrupted by wall bridges consisting of intine and a granular stratum (Fig. 1F) as seen in *Raphionacme palustris* Venter & R. L. Verh. *Camptocarpus* Decne. (Fig. 2A, B) differs from other Periplocoideae genera in that the exine consists of a tectum, granular stratum, and foot layer. The inner walls consist of a granular layer, which on both sides is subtended by a foot layer and intine.

Seven genera of Periplocoideae have pollen coherent into pollinia, rather than tetrads, at anthesis. They are *Decalepis* Wight & Arn., *Epistemma* D. V. Field & J. B. Hall, *Finlaysonia* Wall., *Gymnanthera* R. Br., *Hemidesmus* R. Br., *Schlechterella* K. Schum., and *Streptocaulon* Wight & Arn. In taxa with pollinia, such as *Hemidesmus indicus* (L.)

Schult., each anther produces four free pollinia (Fig. 2C). The pollinia, while in the anther, are not attached to a translator. However, by anthesis the pollinia are shed onto the adhesive-lined receptacle of the translator. The pollinium is not covered by a pollinium wall; it consists of loosely coherent, but not fused, tetrads (Fig. 2D, Table 1). The distal wall exine is smooth and consists of an outer compact stratum (tectum), subtended by a granular stratum. In all genera except *Schlechterella*, the tetrads forming a pollinium exhibit a marked reduction in the number of pores in the distal wall (Fig. 2C; see also numerous SEMs and TEMs in Verhoeven & Venter, 1998a, 1998b). The proximal wall has the same exine stratification as the distal wall. The proximal wall is, however, thinner than the distal wall and pores are also present (Verhoeven & Venter, 1998a). The inner wall, with wall bridges, consists of a tectum, granular stratum, and intine. The exception is *Finlaysonia* Wall., where the inner wall only consists of a granular stratum and intine (Verhoeven & Venter, 1998a).

SECAMONOIDEAE

The Secamonoideae are characterized by having five pollinaria, each of which consists of four pollinia attached to the translator. In *Pervillaea venenata* (Baill.) Klack. the four pollinia are sessile on the translator, and in *Secamone gerrardii* Harv. ex Benth. the four pollinia are attached to a single rather indistinct caudicle (or dorsal process, Kunze, 1993) (Fig. 3A, B). The manner in which the pollinia are attached to the translator is especially variable in this subfamily, and is covered in detail in Civeyrel (1996; Civeyrel & Rowe, 2001 this volume). The pollinium consists of calymmate tetrads, which are coherent but not fused, and is not surrounded by a pollinium wall (Fig. 3C, Table 1). The distal tetrad wall consists of three layers: an outer tectum, well-developed granular layer, and a foot layer subtended by an intine (Fig. 3D). Pores were not observed in either distal or proximal walls. The proximal walls between tetrads consist only of a granular exine layer subtended by an intine (Fig. 3E). The inner walls, separating individual grains of a tetrad from each other, consist of a granular exine layer subtended by an intine (Fig. 3F). The granular exine layer is not continuous but is interrupted by intine bridges (Fig. 3F, arrow).

ASCLEPIADOIDEAE

In the Asclepiadoideae each of the five anthers produces two pollinia, which develop from the two ventral (fertile) pollen sacs. The Asclepiadoideae are thus characterized by having five pollinaria, each of which consists of two pollinia, each attached to the corpusculum by a caudicle (Fig. 4A–D). The pollinium consists of single, inaperturate pollen grains and is surrounded by a pollinium wall (Fig. 4E, F). The pollinia show a great variation in form, varying from oblique-obovate in *Asclepias curassavica* L. (Fig. 4A), hemispherical in *Matelea brevicoronata* (B. L. Rob.) Woodson (Fig. 4B), oblong in *Microloma sagittatum* (L.) R. Br. (Fig. 4C) to reniform in *Stapelia gigantea* N. E. Br. (Fig. 4D). In *Stapelia gigantea*, an extrapollinial appendage, called a wing (El-Gazzar & Hamza, 1973), is present along one side of the pollinium (Fig. 4D). The genus *Fockea* Endl. (Fockeeae), however, differs from the other tribes in that the pollinium consists of calymmate tetrads, which are coherent, but not fused, and is not surrounded by a pollinium wall as shown in *F. angustifolia* K. Schum. and *F. sinuata* (E. Mey.) Druce (Fig. 5A, B, Table 1). The translator consists of a corpusculum, which is clearly divided by a longitudinal cleft. On the ventral part of the corpusculum adhesive material is present, which spreads out to form two lateral adhesive pads (Fig. 5A). The two pollinia are each attached to the upper region of the corpusculum by a short obscure caudicle (or dorsal process, Kunze, 1993). The wall structure of the tetrads forming the pollinium differs as to the distal and proximal walls. The distal wall consists of a tectum (distal exine), thin granular layer, foot layer, and intine as seen in *F. multiflora* K. Schum. (Fig. 5C). The proximal walls between individual tetrads are separated by a space and consist only of a granular exine layer subtended by an intine (Fig. 5D). The inner walls, separating individual grains of a tetrad from each other, consist of a granular exine layer subtended by an intine. The granular layer is not continuous but is interrupted by intine bridges (Fig. 5D). No pores were observed in either distal or proximal walls.

The tribes Marsdenieae, Ceropegieae, and Asclepiadeae in Asclepiadoideae all have a similar pollinium wall structure. The pollinium wall as in *Cynanchum ellipticum* (Harv.) R. A. Dyer consists of a tectum (distal exine layer), a thin granular layer, a foot layer, and intine (Fig. 6A). The tectum (distal exine layer), granular layer, and foot layer together form the ectexine. The granular layer varies from well developed in *Ceropegia stapeliiformis* Haw. (Fig. 6B), to weakly developed in *Gymnema sylvestre* (Retz.) R. Br. ex Schultes (Fig. 6C), where it is present as a discontinuous line. In Asclepiadoideae examined the tectum (distal exine layer) is usually thicker than the foot layer (Fig. 6A). In *Stapelia* L. and *Ceropegia* L. (Ceropegieae)

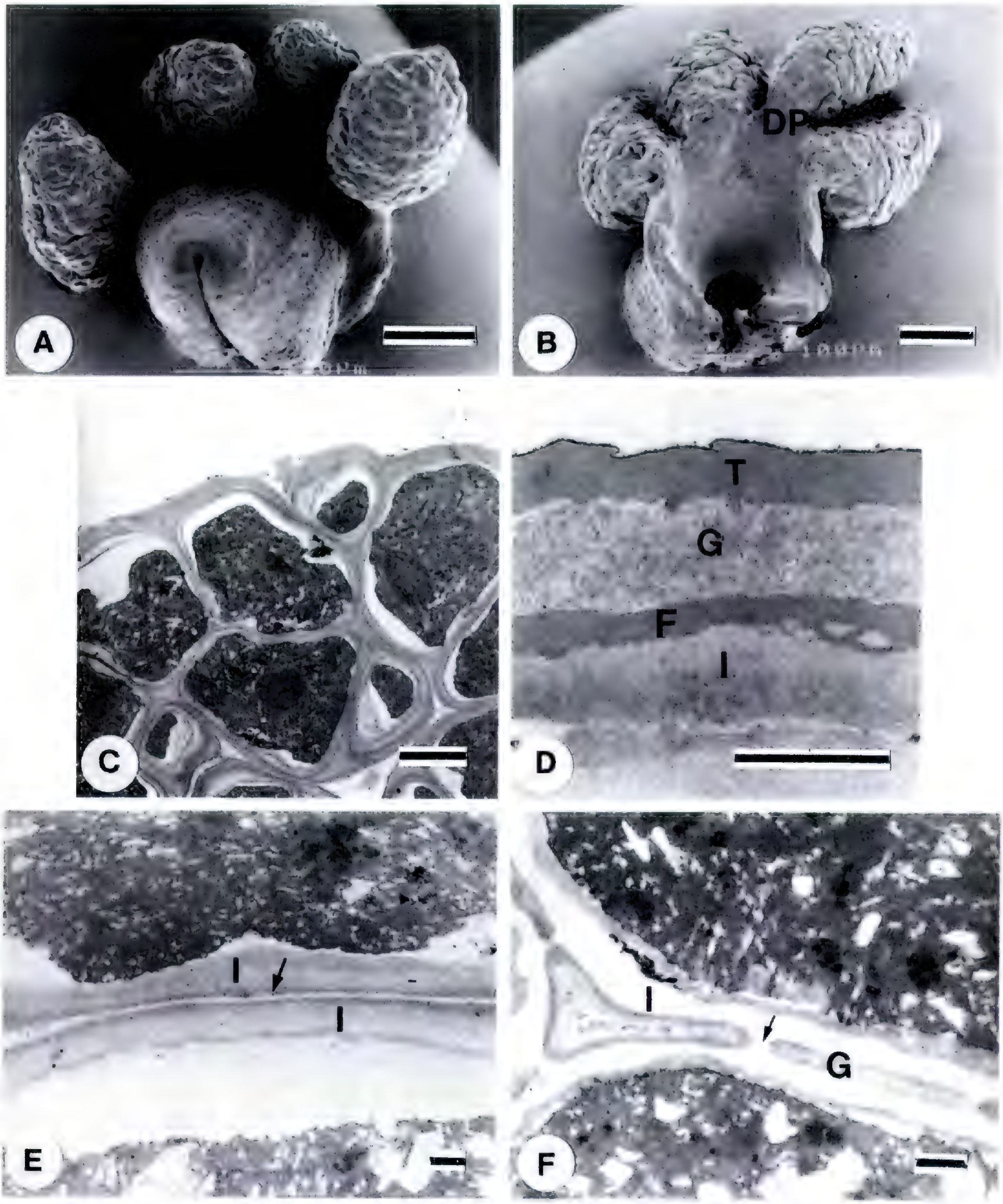


Figure 3. *Secamone gerrardii*. —A. Pollinarium with four pollinia. —B. Pollinarium showing indistinct caudicle (dorsal process) (DP). —C. Section of part of pollinium showing tetrads. —D. Distal tetrad wall showing tectum (T), granular infratectum (G), foot layer (F), and intine (I). —E. Proximal walls between tetrads showing granular exine layer (arrow) subtended by intine (I). —F. Inner wall consisting of granular exine layer (G), intine (I), and wall bridges (arrow). Scale bars: A & B = 100 μm; C–F = 1 μm.

the tectum (distal exine layer) is thinner than the foot layer (Fig. 6B, D). The inner walls separating individual pollen grains are characterized by a granular layer, which forms the separation layer. On each side of the granular layer a foot layer and intine are present as shown here by *Stapelia gigantea* and *Matelea reticulata* (Engelm. ex A. Gray) Woodson (Fig. 6D, E). *Tylophora flanaganii* Schltr. (tribe Asclepiadeae) differs from the other genera investigated. Its pollinium wall consists of columellae and a very thin foot layer, which is subtended by intine (Fig. 6F).

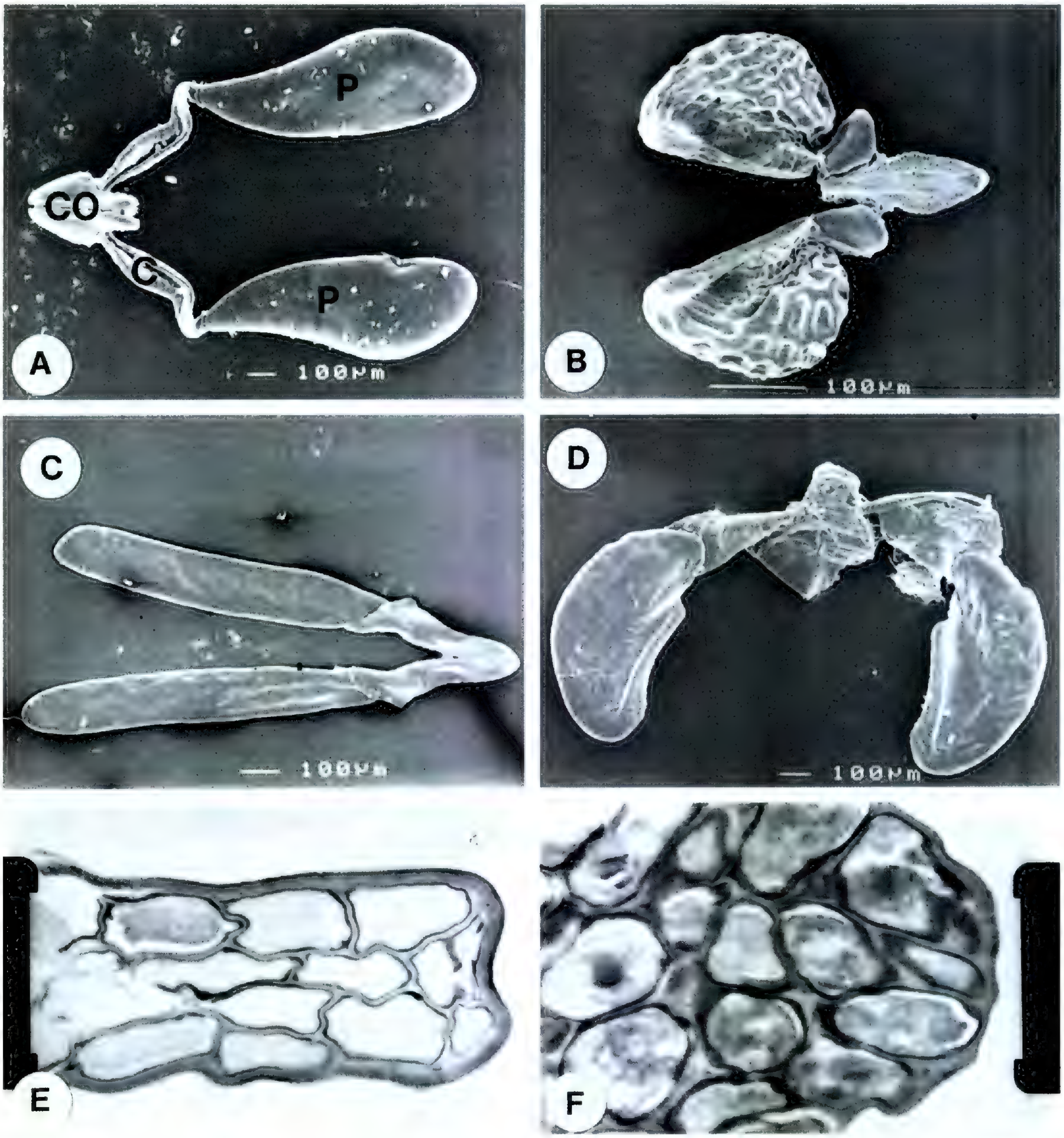


Figure 1. A–D. Pollinaria. —A. *Asclepias curassavica*. —B. *Matelea brevicoronata*. —C. *Microloma sagittatum*. —D. *Stapelia gigantea*. E, F. Section of pollinia showing single pollen grains. —E. *Xysmalobium undulatum*. —F. *Cynanchum ellipticum*. C—caudicle, CO—corpusculum, P—pollinium. Scale bars: A–E = 100 µm; F = 66.6 µm.

Discussion

Tetrad morphology is very similar throughout the Periplocoideae. *Raphionacme* is the only genus that can be distinguished by its multiporate pollen grains in contrast to only 4 to 6 pores in the other genera. Tetrads of *Raphionacme*, having numerous pores, could be regarded as more advanced than genera with 4 to 6 pores (Nilsson et al., 1993), but a detailed phylogenetic analysis would need to be carried out in order to determine this. The exine structure, consisting of a solid stratum (tectum) subtended by a granular stratum, varies little within the Periplocoideae tetrads and pollinia. The inner walls, which separate individual pollen grains of a tetrad, have the same structure as the exterior wall. The walls are, however, thinner and more irregular in appearance than the outer walls. In *Camptocarpus* the structure of the walls differs from the other genera in that the exine consists of a tectum, granular stratum, and foot layer (Verhoeven & Venter, 1994a). The inner walls also differ from the general structure in that the tectum is absent. This inner

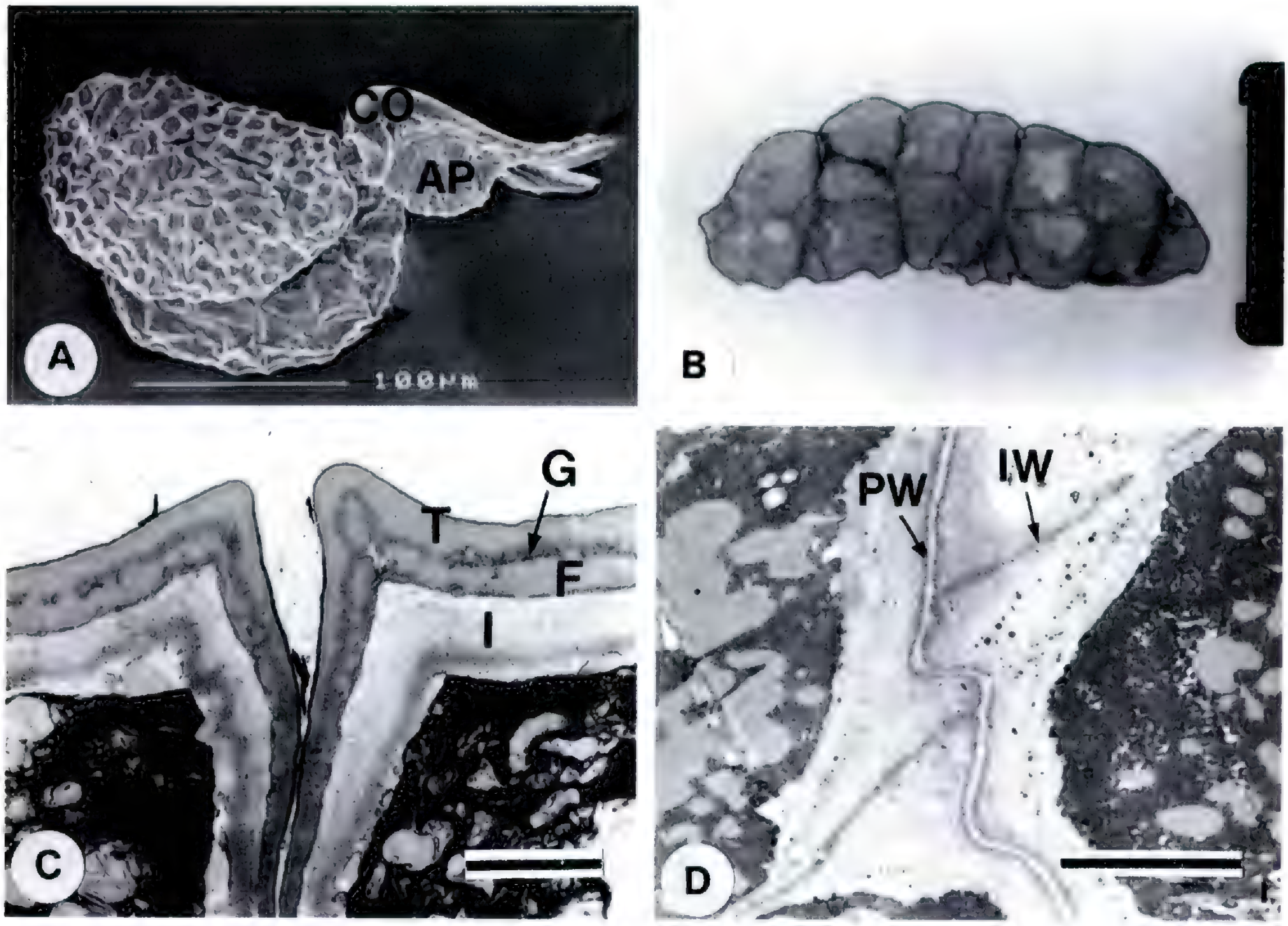


Figure 5. *Fockea.* —A. *F. angustifolia.* Pollinarium showing corpusculum (CO) and adhesive pads (AP). —B. *F. sinuata.* Section of pollinium showing tetrads. —C. *F. multiflora.* Two adjoining tetrads with distal walls showing tectum (T), granular layer (G), foot layer (F), and intine (I). —D. *F. sinuata.* Proximal walls (PW) between tetrads and inner walls (IW). Scale bars: A & B = 100 µm; C = 1 µm; D = 5 µm.

wall, consisting of a granular layer, which on both sides is subtended by a foot layer and intine, is similar to the walls surrounding the individual pollen grains in Asclepiadoideae pollinia. While this would suggest that *Camptocarpus* is palynologically more advanced than the other taxa of Periplocoideae, its floral morphology is otherwise unremarkable (Venter & Verhoeven, 1997; Klackenberg, 1998), as is its position in molecular studies (Civeyrel et al., 1998).

An interesting correlation, which has not received due attention to date, is the reduction in the number of pores in the distal walls, and the presence of pollinia. Whereas in Periplocoideae taxa with pollen as loose tetrads the average number of pores per pollen grain is 4 to 6, in the Asian (and Australian) genera in which the tetrads are coherent into pollinia, the distal wall of the pollen tetrads is inaperturate or only vestigially porate (Civeyrel, 1996). The only genera of Periplocoideae from Africa with pollen tetrads coherent into pollinia are *Schlechterella* K. Schum. and *Epistemma* D. V. Field & J. B. Hall. In contrast to all other pollinia-bearing Periplocoideae, in *Schlechterella* the tetrads that make up a pollinium are multi-porate. The only other genus of Periplocoideae with multi-porate tetrads is *Raphionacme,* suggesting a relationship between these two genera. Indeed *Schlechterella abyssinica* (Chiov.) Venter & R. L. Verh. was previously included in the genus *Raphionacme* (Verhoeven & Venter, 1988) or sometimes as a separate genus *Triodoglossum* Bullock, which is a newer synonym of *Schlechterella.* Results of molecular analyses also suggest a close relationship between *Schlechterella* and *Raphionacme* (Civeyrel et al., 1998). The presence of pollinia composed of multi-porate tetrads in *Schlechterella,* as opposed to nearly inaperturate tetrads in other genera, suggests that pollinia may have arisen twice within Periplocoideae.

Pollinia in Periplocoideae and Secamonoideae are similar in that the pollinium consists of tetrads and is not covered by a pollinium wall, as it is in the Asclepiadoideae (Table 1). However, the wall structure of the tetrads forming the pollinium differs in the two subfamilies. In the Periplocoideae the wall consists of a tectum, granular stratum, and intine. The structures of the distal and proximal walls are morphologically similar; however, the

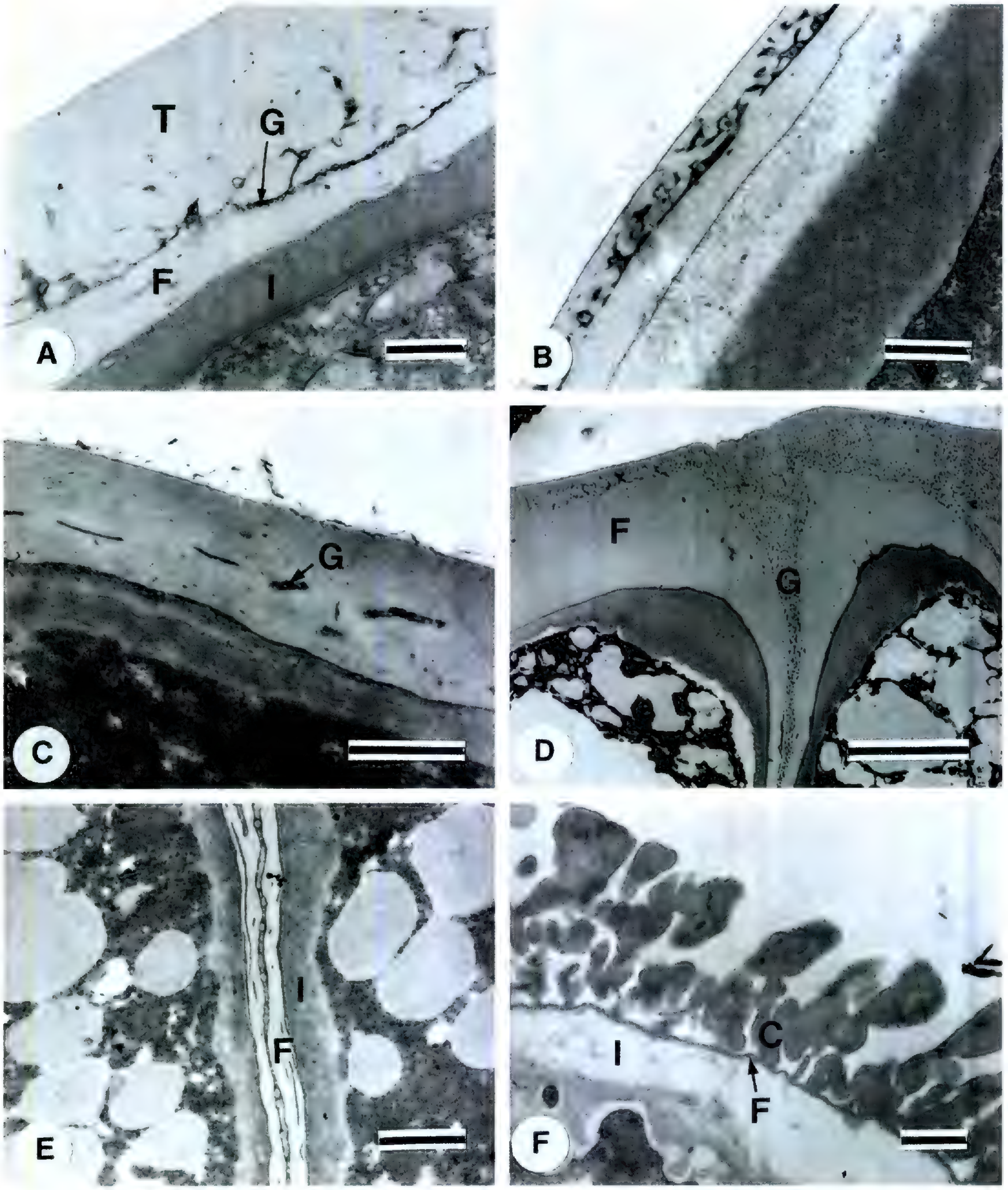


Figure 6. —A. *Cynanchum ellipticum.* Pollinium wall showing tectum (distal exine layer) (T), granular layer (G), foot layer (F), and intine (I). —B. *Ceropegia stapeliiformis.* Pollinium wall showing well-developed granular layer. —C. *Gymnema sylvestre.* Pollinium wall showing weakly developed granular layer (G). —D. *Stapelia gigantea.* Pollinium wall showing thick foot layer (F) and granular layer (G), which forms separation layer between individual pollen grains. —E. *Matelea reticulata.* Inner wall separating individual pollen grains. On each side of the granular layer a foot layer (F) and intine (I) are present. —F. *Tylophora flanaganii.* Pollinium wall showing columellae (C), thin foot layer (F), and intine (I). Scale bars: A–C, E & F = 1 μm; D = 10 μm.

proximal walls are thinner. In the Secamonoideae, as represented here by *Secamone* R. Br., the distal walls have a three-layered exine structure (outer tectum, well-developed granular layer, and a foot layer) subtended by an intine. The outer tectum can also be present as a double layer (Civeyrel, 1995). The proximal walls between tetrads are, however, reduced and only consist of a granular exine layer

subtended by an intine. In the Secamonoideae there is thus a reduction in wall layers in the inner walls, whereas in Periplocoideae the inner walls have all the layers (tectum, granular layer, and intine) and are only thinner than the distal wall. The reduction in inner wall layers in Secamonoideae was also observed by Civeyrel (1995) and Verhoeven and Venter (1994a) in *Pervillaea* Decne. The reduction of wall layers in the inner walls of Secamonoideae is similar to that observed in *Fockea* (Asclepiadoideae, Fockeeae). The reduction in wall layers from Periplocoideae to Secamonoideae supports a more advanced position of the latter at the base of the Asclepiadoideae, as has been found in various independent studies (Sennblad & Bremer, 1996; Civeyrel et al., 1998; Potgieter & Albert, 2001). It is also interesting that the subfamilies Periplocoideae and Secamonoideae as well as the tribe Fockeeae, all regarded as primitive, are restricted to the Old World. In the Periplocoideae pollen mother cell division is of the simultaneous type (Safwat, 1962) just as it is in the Apocynaceae. In *Secamone* the pollen mother cell division is also of the simultaneous type, agreeing in this respect with that of Apocynaceae s. str. and Periplocoideae (Safwat, 1962).

In the Asclepiadoideae the pollinium is covered by a pollinium wall, and single pollen grains, which lack apertures, are present. In the Asclepiadoideae, two successive meiotic divisions produce linear tetrads (Safwat, 1962). The single grains develop as linear tetrads but the cells round off and cannot be identified as tetrads (Dannenbaum & Schill, 1991). The pollinium wall, also referred to as pollinial pellicle (Swarupanandan et al., 1996), envelope (Vijayaraghavan & Shukla, 1976), or ectexine (Dannenbaum & Schill, 1991), consists of a tectum (distal exine layer), thin granular layer, foot layer, and intine. The tectum (distal exine layer), granular layer, and foot layer together form the ectexine. Individual grains are separated from each other by a thin granular layer, which is flanked, on both sides, by the foot layer and intine. *Tylophora flanaganii* in Asclepiadeae differs from the other genera investigated. Instead of a solid distal exine subtended by a granular layer, columellae are present and the foot layer is present as a very thin layer. *Tylophora* R. Br. is a heterogeneous assemblage (Liede, 1996) of about 50 species (Victor et al., 2000). Schill and Jäkel (1978) observed a wide spectrum of pollinium wall sculpturing in *Tylophora*, varying from tectate-perforate, to semitectate, to intectate. At this stage it is not clear how the pollinium structure of *Tylophora flanaganii* relates to that of the other Asclepiadoideae. *Tylophora*, as well as pollinia of other genera of Asclepiadoideae, requires further studies.

The general pollinium structure of the genus *Fockea* differs from that found in other Asclepiadoideae in that the pollinium consists of tetrads and, further, it is not covered by a pollinium wall; in addition, the attachment of pollinium to corpusculum is not by a well-developed caudicle. However, the distal tetrad wall of *Fockea* shows the typical distal pollinium wall structure of Asclepiadoideae (tectum, thin granular layer, and foot layer). The pollinium in *Fockea* is very similar to that in *Secamone* of the subfamily Secamonoideae. In both the pollinium consists of tetrads and is not covered by a pollinium wall, and in both the inner walls are reduced. The pollinia in the two genera differ only in the structure of the distal wall in that the granular layer is well developed in Secamonoideae and thin in *Fockea* (Table 1). All these characters support a position for *Fockea* intermediate between Secamonoideae and the more advanced Asclepiadoideae. This position is supported by independent studies of translator ontogeny (Kunze, 1993) as well as molecular phylogeny obtained from sequences of both *mat*K (Civeyrel et al., 1998) and *trn*L (Potgieter & Albert, 2001).

Nilsson (1990) studied pollen of *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don, pointing out the similarity between pollen grains of *Holarrhena* (Apocynaceae: Plumerioideae) and those of certain taxa of the Apocynaceae: Apocynoideae, e.g., *Alafia*, *Pleioceras*. Later *Holarrhena* and related genera (subtribe Holarrheninae) were transferred to the Apocynoideae for several reasons, including corolla lobe aestivation, differentiation of anthers, pollen morphology, relationship between gynoecium and androecium, comose seeds, and secondary chemistry, by Endress et al. (1990). The exine in *Holarrhena* and *Alafia* (also in Apocynoideae) consists of a tectum subtended by a granular stratum reminiscent of the exine structure in the Periplocoideae. In *Kopsia flavida* Blume (Rauvolfioideae) Nilsson (1990) observed an exine subdivided into two strata by a discontinuous line of osmiophilic substance. This exine structure is very similar to the pollinium wall of the Asclepiadoideae. Based on a number of phylogenetic reconstructions, these similarities must, however, be considered as parallelisms.

In the family Apocynaceae s.l., there is a phylogenetic line of development that is reflected in pollen structure. The evolutionary trends that can be followed are 3- or 4-colporate grains (Rauvolfioideae), which are more primitive than the 3-porate grains in Apocynoideae. Tetrads consisting of loosely attached grains in *Apocynum* L. s.l. (Nilsson et al., 1993) (Apocynoideae) can be regarded as

more advanced than 3-porate grains. Within the Periplocoideae the tetrads of *Raphionacme*, having numerous pores, could be regarded as more advanced than tetrads of genera with 4 to 6 pores (Nilsson et al., 1993). Pollinia (observed in 7 genera of the Periplocoideae: *Decalepis*, *Epistemma*, *Finlaysonia*, *Gymnanthera*, *Hemidesmus*, *Schlechterella*, and *Streptocaulon*) can be regarded as more advanced than single tetrads. Genera with pollinia, however, do not group in the same clade (Venter & Verhoeven, 2001). Genera with pollinia are also not necessarily more advanced in flower structure. *Hemidesmus* shows the most advanced condition in the valvate corolla lobe aestivation, anthers postgenitally fused into a ring, and the staminal tube (Nilsson et al., 1993; Endress & Bruyns, 2000). Of the Periplocoideae with pollinia, the multi-porate tetrads in the pollinium of *Schlechterella* differ significantly from the nearly inaperturate tetrads in the pollinia of all other taxa studied. This suggests that pollinia may have arisen twice within the Periplocoideae: once in Asia, and once in Africa.

If the type of pollinium and translator (which together form the pollinarium) in Asclepiadoideae are considered the most advanced, trends of development in that direction can be observed beginning already in the Apocynaceae s. str., especially *Apocynum*, in which tetrads are shed onto a simple sticky band-like translator (Nilsson et al., 1993). In the Periplocoideae the pollinia are free, consist of tetrads, and the distal walls are inaperturate or only vestigially porate. Pores are, however, present in proximal walls. Correlated with the appearance of an inaperturate distal wall is a reduction in proximal wall thickness. In the Secamonoideae, where the pollinarium has four pollinia, the pollinia are attached to a translator, which varies from consisting of only a corpusculum in *Pervillaea* (Omlor, 1996; Klackenberg, 1995a, b), to corpusculum with weakly developed caudicles in *Secamone* (Civeyrel, 1995), to corpusculum and two well-developed caudicles in *Secamonopsis* (Omlor, 1996; Civeyrel & Rowe, 2001). The pollinium in Secamonoideae consists of tetrads and is not covered with a pollinium wall. The tetrads are inaperturate and the proximal walls are reduced in thickness and wall layers. In *Fockea* (Fockeeae, Asclepiadoideae) the pollinium is similar to those in the Periplocoideae and Secamonoideae in that it consists of tetrads and is not covered with a pollinium wall. The pollinia are, however, reduced to two per pollinarium. *Fockea* also shows a similarity with the Secamonoideae (except *Secamonopsis*) in that the pollinia are not attached to distinct caudicles, but are nearly sessile on a single dorsal plate (Kunze, 1993). The two pollinia per pollinarium are, however, characteristic for the Asclepiadoideae. The exine structure in *Fockea* is also very similar to that found in the rest of the Asclepiadoideae.

The presence of all the wall layers in proximal and inner walls, together with the tetrads coherent into a pollinium that does not become attached to a translator during ontogeny, could be interpreted as support for an independent evolution of pollinia in the Periplocoideae from that in Secamonoideae and Asclepiadoideae. It is also interesting to note that the individual grains in tetrads of *Camptocarpus* (Periplocoideae), as well as the single grains of *Kopsia flavida*, have an exine wall structure very similar to that of the individual pollen grains in the Asclepiadoideae pollinium, showing that the genetic information for this type of wall structure is also present in the more primitive groups.

Literature Cited

Civeyrel, L. 1994. Variation and evolution of pollen types in the genus *Secamone* (Asclepiadaceae, Secamonoideae). Compt. Rend. Acad. Sci. Paris 317: 1159–1165.

———. 1995. Pollen morphology and ultrastructure of the genus *Secamone* in Africa. Pp. 207–215 *in* A. Le Thomas & E. Roche (editors), 2nd Symposium on African Palynology, Tevuren, Belgium. CIFEG, Orleans.

———. 1996. Phylogénie des Asclepiadaceae, Approche Palynologique et Moléculaire. Unpublished Ph.D. Thesis, Université Montpellier II.

——— & N. Rowe. 2001. Phylogenetic relationships of Secamonoideae based on the plastid gene *mat*K, morphology, and biomechanics. Ann. Missouri Bot. Gard. 88: 583–602.

———, A. Le Thomas, K. Ferguson & M. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *mat*K sequences. Molec. Phylogenet. Evol. 9: 517–527.

Dannenbaum, C. & R. Schill. 1991. Die Entwicklung der Pollentetraden und Pollinien bei den Asclepiadaceae. Biblioth. Bot. 141: 1–138.

El-Gazzar, A. & M. K. Hamza. 1973. Morphology of the twin pollinia of Asclepiadaceae. Pollen & Spores 15: 459–470.

———, ——— & A. A. Badawi. 1974. Pollen morphology and taxonomy of Asclepiadaceae. Pollen & Spores 16: 227–239.

Endress, M. E. & P. V. Bruyns. 2000. A revised classification of the Apocynaceae s.l. Bot. Rev. (Lancaster) 66: 1–56.

———, M. Hesse, S. Nilsson, A. Guggisberg & J. Zhu. 1990. The systematic position of the Holarrheninae (Apocynaceae). Pl. Syst. Evol. 171: 157–185.

Endress, P. K. 1994. Diversity and Evolutionary Biology of Tropical Flowers. Cambridge Univ. Press, Cambridge.

Galil, J. & M. Zeroni. 1969. On the organization of the pollinium in *Asclepias curassavica*. Bot. Gaz. 130: 1–4.

Klackenberg, J. 1995a. Malagasy Asclepiadaceae: Reinstatement of the genus *Pervillaea* and two new combinations. Phytologia 78: 189–191.

———. 1995b. Revision of the Malagasy genus *Perrillaea* (Asclepiadaceae) and its phylogenetic relationships to *Calyptranthera*. Nordic J. Bot. 16: 165–184.
———. 1998. Taxonomy and phylogeny of the genus *Camptocarpus* s.l. (Periplocoideae, Asclepiadaceae). Bot. Jahrb. Syst. 120: 45–85.
———. Notes on Secamonoideae (Apocynaceae) in Africa. Adansonia 23(2) (in press).
Kunze, H. 1993. Evolution of the translator in Periplocaceae and Asclepiadaceae. Pl. Syst. Evol. 185: 99–122.
———. 1994. Ontogeny of the translator in Asclepiadaceae s. str. Pl. Syst. Evol. 193: 223–242.
Liede, S. 1996. Anther differentiation in the Asclepiadaceae–Asclepiadeae: Form and function. Pp. 221–235 *in* W. G. D'Arcy & R. C. Keating (editors), The Anther: Form, Function and Phylogeny. Univ. Press, Cambridge.
———. 1997. Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae)—A synopsis. Taxon 46: 233–247.
——— & F. Albers. 1994. Tribal disposition of genera in the Asclepiadaceae. Taxon 43: 201–231.
Nilsson, S. 1990. Taxonomic and evolutionary significance of pollen morphology in the Apocynaceae. Pl. Syst. Evol., Suppl. 5: 91–102.
———, M. E. Endress & E. Grafström. 1993. On the relationship of the Apocynaceae and Periplocaceae. Grana, Suppl. 2: 3–20.
Omlor, R. 1996. Do *Menabea venenata* and *Secamonopsis madagascariensis* represent missing links between Periplocaceae, Secamonoideae and Marsdenieae (Asclepiadaceae)? Kew Bull. 51: 695–715.
Potgieter, K. & V. A. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.
Safwat, F. M. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. Ann. Missouri Bot. Gard. 49: 95–129.
Schick, B. 1982. Zur Morphologie, Entwicklung, Feinstruktur und Funktion des Translators von *Periploca* L. (Asclepiadaceae). Trop. Subtrop. Pflanzenwelt 40: 513–553.
Schill, R. & U. Jäkel. 1978. Beitrag zur Kenntnis der Asclepiadaceen-Pollinarien. Trop. Subtrop. Pflanzenwelt 22: 1–122.
Semmblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.
Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. C. Basha. 1996. The subfamilial and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.
Venter, H. J. T. & R. L. Verhoeven. 1997. A tribal classification of the Periplocoideae (Apocynaceae). Taxon 46: 705–720.
——— & ———. 2001. Diversity and relationships within the Periplocoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 550–568.
Verhoeven, R. L. & H. J. T. Venter. 1988. Pollen morphology of *Raphionacme* (Periplocaceae). S. African J. Bot. 54: 123–132.
——— & ———. 1993. Pollen morphology of *Curroria*, *Mondia*, *Socotranthus* and *Stomatostemma* (Periplocaceae). Bothalia 23: 105–110.
——— & ———. 1994a. Pollen morphology of the Periplocaceae from Madagascar. Grana 33: 295–308.
——— & ———. 1994b. Pollen morphology of *Periploca* (Periplocaceae). S. African J. Bot. 60: 198–202.
——— & ———. 1997. The translator of *Raphionacme* (Periplocaceae). S. African J. Bot. 63: 46–54.
——— & ———. 1998a. Pollinium structure in Periplocoideae (Apocynaceae). Grana 37: 1–14.
——— & ———. 1998b. Pollinium in *Schlechterella*: Periplocoideae (Apocynaceae). S. African J. Bot. 64: 256–257.
———, ——— & J. D. S. Kotze. 1989. Pollen morphology of *Petopentia* and *Tacazzea* (Periplocaceae). S. African J. Bot. 55: 207–214.
Victor, J. E., C. L. Bredenkamp, H. J. T. Venter, P. V. Bruyns & A. Nicholas. 2000. Apocynaceae. Pp. 71–98 *in* O. A. Leistner (editor), Seed Plants of Southern Africa: Families and Genera. Strelitzia 10. National Botanical Institute, Pretoria.
Vijayaraghavan, M. R. & A. K. Shukla. 1976. The nature of covering around the aggregate of microspores in *Pergularia daemia* (Forsk.) McC. & Blat. Ann. Bot. (Oxford), n.s. 40: 417–421.

Appendix 1. Taxa examined.

Taxon	Voucher information
Periplocoideae	
Baroniella camptocarpoides Costantin & Gallaud	Verhoeven & Venter (1994a)
Baroniella linearis (Choux) Bullock	Verhoeven & Venter (1994a)
Baseonema camptocarpoides Choux	Verhoeven & Venter (1994a)
Baseonema lineare Choux	Verhoeven & Venter (1994a)
Camptocarpus acuminatus (Choux) Venter [as *Tanulepis acuminata* (Choux) Choux]	Verhoeven & Venter (1994a)
Camptocarpus linearis Decne. [as *Tanulepis linearis* (Decne.) Choux	Verhoeven & Venter (1994a)
Camptocarpus mauritianus (Lam.) Decne. [as *C. bojeri* Jum. & H. Perrier]	Verhoeven & Venter (1994a)
Camptocarpus semihastatus (Decne.) Klack. [as *Harpanema acuminatum* Decne.]	Verhoeven & Venter (1994a)
Camptocarpus sphenophyllus (Baker) Venter [as *Tanulepis sphenophylla* Balf. f. ex Baker]	Verhoeven & Venter (1994a)
Cryptolepis albicans Jum. & H. Perrier	Verhoeven & Venter (1994a)
Cryptolepis grandidieri Roxb. ex R. Br.	Verhoeven & Venter (1994a)
Cryptostegia grandiflora R. Br.	Verhoeven & Venter (1994a)
Cryptostegia madagascariensis Bojer ex Decne.	Verhoeven & Venter (1994a)
Curroria decidua Planch. ex Benth.	Verhoeven & Venter (1993)
Curroria macrophylla A. R. Smith	Verhoeven & Venter (1993)
Curroria migiurtina (Chiov.) Bullock	Verhoeven & Venter (1993)
Curroria volubilis (Schltr.) Bullock	Verhoeven & Venter (1993)
Decalepis arayalpathra (J. Joseth & V. Chandras) Venter	Verhoeven & Venter (1998a)
Decalepis hamiltonii Wight & Arn.	Verhoeven & Venter (1998a)
Decalepis nervosa (Wight & Arn.) Venter	Verhoeven & Venter (1998a)
Decalepis salicifolia Bedd. ex Benth. & Hook. f. [as *Utleria salicifolia* Bedd. ex Benth. & Hook. f.]	Verhoeven & Venter (1998a)
Finlaysonia curtisii (King & Gamble) Venter [as *Gongylosperma curtisii* King & Gamble]	Verhoeven & Venter (1998a)
Finlaysonia insularum (King & Gamble) Venter [as *Meladerma insularum* King & Gamble	Verhoeven & Venter (1998a)
Finlaysonia khasiana (Kurz) Venter [as *Stelmacrypton khasianum* (Kurz) Baill.]	Verhoeven & Venter (1998a)
Finlaysonia maritima Backer ex K. Heyne	Verhoeven & Venter (1998a)
Finlaysonia obovata Wall.	Verhoeven & Venter (1998a)
Finlaysonia pierrei (Costantin) Venter [as *Atherolepis pierrei* Costantin]	Verhoeven & Venter (1998a)
Gonocrypta grevei Baill.	Verhoeven & Venter (1994a)
Gymnanthera nitida R. Br.	Verhoeven & Venter (1998a)
Harpanema acuminatum Decne.	Verhoeven & Venter (1994a)
Hemidesmus indicus (L.) Schult.	Verhoeven & Venter (1998a)
Ischnolepis tuberosa Jum. & H. Perrier	Verhoeven & Venter (1994a)
Mondia ecornuta (N. E. Br.) Bullock	Verhoeven & Venter (1993)
Mondia whitei (Hook. f.) Skeels	Verhoeven & Venter (1993)
Pentopetia androsaemifolia Decne.	Verhoeven & Venter (1994a)
Periploca acuminata Rahman & Wilcock	Verhoeven & Venter (1994b)
Periploca aphylla Decne.	Verhoeven & Venter (1994b)
Periploca graeca L.	Verhoeven & Venter (1994b)
Periploca sepium Bunge	Verhoeven & Venter (1994b)
Periploca visciformis (Vatke) Schum.	Verhoeven & Venter (1994b)
Petopentia natalensis (Schltr.) Bullock	Verhoeven et al. (1989)
Raphionacme abyssinica Chiov.	Verhoeven & Venter (1998a)
Raphionacme hirsuta (E. Mey.) R.A. Dyer	Verhoeven & Venter (1988)
Raphionacme madiensis S. Moore	Verhoeven & Venter (1988)
Raphionacme palustris Venter & R. L. Verh.	Verhoeven & Venter (1988)
Schlechterella africana (Schltr.) K. Schum.	Verhoeven & Venter (1998b)

Appendix 1. Continued.

Taxon	Voucher information
Streptocaulon baumii Decne.	Verhoeven & Venter (1998a)
Streptocaulon cumingii (Turcz.) Vill.	Verhoeven & Venter (1998a)
Streptocaulon extensum Wight	Verhoeven & Venter (1998a)
Streptocaulon juventas (Lour.) Merr. [as *S. griffithii* Hook. f.] [as *S. tomentosum* Wight]	Verhoeven & Venter (1998a)
Streptocaulon kleinii Wight & Arn.	Verhoeven & Venter (1998a)
Streptocaulon sylvestre Wight	Verhoeven & Venter (1998a)
Tacazzea apiculata Oliv.	Verhoeven et al. (1989)
Tanulepis acuminata Choux	Verhoeven & Venter (1994a)
Tanulepis linearis (Decne.) Choux	Verhoeven & Venter (1994a)
Tanulepis sphenophylla Balf. f.	Verhoeven & Venter (1994a)
Secamonoideae	
Pervillea venenata Baill. [as *Menabea venenata* Baill.]	Verhoeven & Venter (1994a)
Secamone gerrardii Harv. ex Benth.	SOUTH AFRICA. KwaZulu-Natal: Eshowe, 27/10/1962, *H. J. T. Venter 645* (BLFU). SOUTH AFRICA. Northern Province: Soutpansberg, 20/10/1984, *N. Jacobson 3409* (PRE).
Asclepiadoideae, Fockeeae	
Fockea angustifolia K. Schum.	SOUTH AFRICA. Mpumalanga: Marble Hall, 27/11 1972, *Vorster & Jackson 2160* (PRE).
Fockea multiflora K. Schum.	NAMIBIA. Okaruwizu, 08/10/1960, *Giess & Wiss 3310* (PRE).
Fockea sinuata (E. Mey.) Druce	SOUTH AFRICA. Northern Cape: Britstown, 11/03/1988, *L. Smook 6854* (PRE).
Asclepiadoideae, Marsdenieae	
Dregea macrantha Klotzsch	NAMIBIA. Etosha Game Park, No date, *K. Tilney 1131* (PRE).
Gymnema sylvestre (Retz.) R. Br. ex Schultes	SOUTH AFRICA. Northern Province: Messina, 08/01/1974, *G. K. Theron 2965* (PRE).
Asclepiadoideae, Ceropegieae	
Caralluma speciosa N. E. Brown	TANZANIA. Lake Manyara National Park, 25/03/1968, *Greenway & Kanuri 13244* (PRE).
Ceropegia stapeliiformis Haw.	SOUTH AFRICA. Eastern Cape: Jansenville, /12/1953, *B. Stevens 4* (BLFU).
Hoodia gordonii (Masson) Sweet ex Decne.	NAMIBIA. Ochta, 24/10/1981, *H. J. T. Venter 8628* (BLFU).
Stapelia gigantea N. E. Br.	SOUTH AFRICA. KwaZulu-Natal: Mtunzini, 10/03/1965, *H. J. T. Venter 1830* (BLFU).
Asclepiadoideae, Asclepiadeae	
Asclepias curassavica L.	SOUTH AFRICA. Free State: Bloemfontein, -/05/1993, *R. L. Verhoeven 614* (BLFU).
Cynanchum ellipticum (Harv.) R. A. Dyer	SOUTH AFRICA. KwaZulu-Natal: Mtubatuba, 20/05/1968, *H. J. T. Venter 4749* (BLFU).
Glossonema revoilii Franch.	ETHIOPIA. Road Dagahour to Jijiga, 19/03/1941, *O. West 5433* (PRE).
Matelea brevicoronata (B. L. Rob.) Woodson	U.S.A. Texas, *Prinzie 218* (MO).
M. reticulata (Engelm. ex A. Gray) Woodson	U.S.A. Texas, *Prinzie 230* (MO).
Microloma sagittatum (L.) R. Br.	SOUTH AFRICA. Western Cape: Vredendal, 08/08/1977, *A. leRoux 2155* (PRE).
Pentarrhinum insipidum E. Mey.	SOUTH AFRICA. Mpumalanga: Middelburg, 16/10/1968, *G. K. Theron 1875* (PRE).
Tylophora flanaganii Schltr.	SOUTH AFRICA. KwaZulu-Natal: Port Shepstone, 27/06/1967, *R. Strey 5789* (PRE).
Xysmalobium undulatum (L.) W. T. Aiton	SOUTH AFRICA. Gauteng: Randburg, 01/11/1976, *L. Liebenberg 8528* (PRE).

PHYLOGENETIC RELATIONSHIPS OF SECAMONOIDEAE BASED ON THE PLASTID GENE *mat*K, MORPHOLOGY, AND BIOMECHANICS[1]

Laure Civeyrel[2] and Nick Rowe[3]

ABSTRACT

Relationships between Secamonoideae and their closest relatives in Apocynaceae, as well as genera and species within the subfamily, have been examined, using a molecular phylogeny with the plastid gene *mat*K. Our molecular results show that Secamonoideae form a monophyletic group. Delimitations of genera are also discussed together with the evolution of pollinarium and biomechanical characters within species. The patterns of pollinial organization, as well as change in growth forms, are mapped onto a phylogenetic tree obtained from the plastid gene *mat*K, all of which together significantly contribute toward a better understanding of the evolution of the Apocynaceae s.l.

Key words: Apocynaceae, biomechanics, *mat*K, palynology, phylogeny, pollinia, Secamonoideae.

The Secamonoideae (Endlicher, 1838) are the smallest subfamily of the former Asclepiadaceae sensu stricto with only 7 widely accepted genera and less than 200 species. It is the sister group of the Asclepiadoideae, which are by far the largest subfamily of the Apocynaceae s.l. There is currently little phylogenetic information about Secamonoideae (Safwat, 1962; Nicholas & Baijnath, 1994; Civeyrel, 1996; Klackenberg, 1996b; Omlor, 1996; Sennblad & Bremer, 1996; Klackenberg, 1997; Civeyrel et al., 1998). This paper uses additional data to examine relationships between Secamonoideae and their closest relatives, and focuses on the relationships between genera and species within the subfamily itself. In addition, the study presents observations on pollinarium characters as well as biomechanical changes in growth form within the group.

The systematic position of Secamonoideae based on morphological characters is complicated because some characters appear to link them more closely to basal Apocynaceae s. str. and Periplocoideae than to Asclepiadoideae (Omlor, 1996). Many characters have been proposed for delimitation of the Secamonoideae, but they frequently occur outside the subfamily as well. For example, Secamonoideae have simultaneous microsporogenesis (Safwat, 1962) together with pollen in tetrads, which are rectangular or T-shaped and similar to the few basal Apocynaceae with pollen in tetrads, as well as the tetrads found in Periplocoideae (Nilsson et al., 1993; Verhoeven & Venter, 1993, 1994, 1998, 2001 this volume). In contrast, apomorphic Asclepiadoideae have successive microsporogenesis and linear tetrads (Safwat, 1962; Puri & Shiam, 1966; Bruyns & Forster, 1991). Nevertheless, pollen in rectangular tetrads has also been described in the genus *Fockea* (Asclepiadoideae) (Civeyrel, 1996), although details concerning microsporogenesis for this genus are lacking. As in all Apocynaceae (Endress et al., 1983), a structurally defined compitum has been found in Secamonoideae. A

[1] We thank Mary Endress and Doug Stevens for the invitation to participate in the symposium "Evolution and Phylogenetics of Apocynaceae s.l." at the XVI International Botanical Congress. We also thank the director of the Parc Zoologique et Botanique de Tsimbazaza and the director of ANGAP in Madagascar. We are particularly grateful to Mrs. Faramalala, Mrs. Rakotovao, Mr. Rapanarivo, Mr. Rafamantanansoa, and Mr. Ranaivojaona for the help they provided during the two field seasons in Madagascar (1994 & 1999). This research was supported by a grant from the European Community in the program Human Capital and Mobility (ERBCHBICT-930564) for the field trip of 1994, and by the Percy Sladen fund (Linnean Society, London) for 1999 to L. Civeyrel. We thank Mark Chase, Tony Cox, and John Freudenstein for support and advice; Anette de Bruijn for technical assistance in the laboratory of Molecular Systematics at Kew; and Samuel Bonin for assistance in the field.

[2] Laboratoire d'Ecologie Terrestre, Bat IV R3, 118 route de Narbonne, UPS, Toulouse 31062 cedex 4, France. civeyrel@ciet.fr.

[3] Botanique et bioinformatique de l'architecture des plantes, UMR 5120, Boulevard de la Lironde–TA40/PS2, 34398 Montpellier, France. rowe@isem.univ-montp2.fr.

compitum is the unified pollen tube transmitting tract, which allows the distribution of pollen tubes. In the Apocynaceae, where the overwhelming majority of species are apocarpous, a special type of compitum is formed through the postgenital fusion of the carpel apices, allowing pollen that has been deposited only on one side to be distributed to both carpels (or in the case of the higher pollinium-bearing Apocynaceae, a single inserted pollinium to distribute pollen tubes into both ovaries) (Endress et al., 1983; Kunze, 1991). Secamonoideae have been reported to lack a true style (Swarupanandan et al., 1996), but there is a true style above the carpels in *Pervillaea* and *Calyptranthera*, both of which belong to this subfamily (Omlor, 1996; Klackenberg, 1997). Upwardly directed, sterile placental margins are found in *Secamone*, but also in *Mandevilla* (basal Apocynaceae), whereas in most basal Apocynaceae, Periplocoideae, and Asclepiadoideae the sterile margins are directed downward (Woodson & Moore, 1938; Safwat, 1962; Nicholas & Baijnath, 1994). A critical reexamination of Brown's (1810) palynological characters has led to the proposal of a new set of diagnostic pollinium characters (Civeyrel et al., 1998), which, at present, are the only characters that reliably define the subfamily Secamonoideae. Members of the Secamonoideae can be distinguished from Periplocoideae and Asclepiadoideae by "having 20 pollinia, with their inner walls reduced, and which are connected to a translator apparatus composed of a corpusculum and one (or rarely two) caudicula, in addition to various degrees of staminal synorganization" (Civeyrel et al., 1998: 523). Pollinarium characters, especially the combination of number of pollinia and the way they are attached to the translator apparatus, remain the most valuable characters to distinguish this group. In Asclepiadoideae there are only 10 pollinia, which become attached to the caudiculae of the translator apparatus during ontogeny, whereas in Periplocoideae the 20 pollinia, when they are present, are not attached to the translator apparatus via caudiculae, but are shed onto it, which is very distinctive.

The Secamonoideae, which contain 7 generally recognized genera (*Secamone*, *Toxocarpus*, *Genianthus*, *Pervillaea*, *Secamonopsis*, *Calyptranthera*, and *Trichosandra*) and under 200 species, are restricted to the Old World tropics. There are also two genera of uncertain taxonomic position, i.e., *Goniostemma* and *Schistocodon*. The monotypic African genus *Rhynchostigma* has recently been put into synonymy under *Secamone* (Klackenberg, in press). *Secamone* is the largest genus with more than 80 species, which occur mainly in Madagascar (Klackenberg, 1992a), Africa (Goyder, 1992), and Asia (Forster & Harold, 1989; Klackenberg, 1992b). *Toxocarpus* with almost 40 species occurs mainly in Asia, as does *Genianthus* with 16 Asiatic species (Klackenberg, 1995a). The other four genera, *Pervillaea* (Klackenberg, 1995b), *Secamonopsis* (Civeyrel & Klackenberg, 1996), *Calyptranthera* (Klackenberg, 1996a; Klackenberg, 1997), and *Trichosandra* (Friedman, 1990) are restricted to Madagascar or to the Mascarene Islands, with less than 10 species each. The main center of endemism is Madagascar, where half of the known species and genera occur, followed by southeast Asia and Africa.

Malagasy genera of Secamonoideae, e.g., *Secamone*, especially the *S. cristata* group (*S. cristata* and its four subspecies, as well as *S. bosseri* and *S. polyantha*), *Pervillaea*, and *Secamonopsis*, show a remarkable range of growth habits, from erect to partially procumbent, small-bodied shrubs to larger-bodied twining lianas. Phylogenetic analysis of Secamonoideae offers the opportunity to analyze changes in growth habit during the evolutionary radiation of this group of plants in Madagascar, particularly with reference to changes from lianoid to shrubby habits. The former Asclepiadaceae are a predominantly lianoid family, but previous analyses have demonstrated reversals from lianoid growth forms to self-supporting habits (Civeyrel, 1996). Biomechanical and anatomical studies have been recently carried out to characterize different plant growth forms and to critically assess the developmental characters that underlie changes in stem mechanics (Rowe & Speck, 1998; Speck & Rowe, 1999). For the Secamonoideae we were interested in changes in growth form, in particular transitions from climbing forms to self-supporting species. We also wanted to see whether the mechanics and underlying anatomical development would be similar for lianas of different species, and how historical developmental constraints might have influenced the evolution of growth forms. Our initial investigation of the Secamonoideae presented here illustrates the differences in biomechanical behavior between two species of *Secamonopsis* also included in the phylogenetic analysis, with one represented by a self-supporting shrub and the other a twining liana. Our preliminary analysis represents a basis for examining the developmental homologies underlying the lianescent growth forms within the group and for investigating those sporadic switches to self-supporting growth forms within a predominantly lianoid group.

The Secamonoideae also show a range of interesting synorganizations in their flowers. In the former Asclepiadaceae, there is an unusual synorganization between floral parts and also between organs of different categories (Endress, 1990, 1996). Endress (1990) has described synorganization as the intimate structural connection of two or several neighboring structures to form a functional system or apparatus. In Secamonoideae there is a special kind of synorganization that occurs between pollinia and the translator apparatus, as well as within pollinia (Civeyrel, 1994, 1996). The pollinarium of Secamonoideae is composed of four pollinia connected to the translator apparatus, which in turn is made up of a corpusculum and one or two caudicula. Two caudicula have been observed in *Secamone* (Civeyrel, 1994), *Genianthus* (Civeyrel, 1996), and *Secamonopsis* (Civeyrel, 1996; Civeyrel & Klackenberg, 1996; Omlor, 1996). The four pollinia belonging to one pollinarium are each derived from a different pollen sac coming from one theca each of two adjacent anthers and are attached to the translator apparatus, which is secreted by the stigma head. This is the most common form of synorganization found in most taxa of the former Asclepiadaceae (Fig. 1A). In some taxa of the Secamonoideae there is, additionally, a special type of synorganization between pollinia from the same anther (intrapollinial synorganization) (Fig. 1B), as well as synorganization between pollinia from adjacent anthers (interpollinial organization; Fig. 1C). Synorganization within pollinia of an anther, and especially this special type of synorganization of pollinia from the pollen sacs of different anthers constitute the only record of this sort of synorganization in the angiosperms.

THE PLASTID GENE *mat*K

Systematists use cladistic analyses to study relationships among taxa but also to observe character evolution (Sibley & Ahlquist, 1987; Mickevich & Weller, 1990). Changes in morphological characters on a cladogram may also be evaluated simply by mapping characters onto molecular phylogenies, or observed directly in analyses combining molecular and morphological characters. One gene frequently used in phylogenetic reconstruction in recent years has been *mat*K (Steele & Vilgalys, 1994; Johnson & Soltis, 1994, 1995; Johnson et al., 1996; Liang & Hilu, 1996; Plunkett et al., 1996; Soltis et al., 1996; Hilu & Liang, 1997; Manos, 1997; Plunkett et al., 1997; Sang et al., 1997; Matsumoto et al., 1998; Xiang et al., 1998; Hilu & Alice, 1999; Kron et al., 1999; Les et al., 1999; Li et al., 1999; Thiv et al., 1999; Wang et al., 1999; Yokoyama et al., 2000) because of its suitable rate of mutation and resolution for infrafamilial relationships. The plastid gene *mat*K (Liere & Link, 1995; Neuhaus & Link, 1987; Sugita et al., 1985; Wolfe, 1991; Wolfe et al., 1991, 1992) is a single-copy gene of approximately 1530 base pairs in length, situated in the large single-copy region of the chloroplast. The plastid gene *mat*K has been previously used to assess the complex relationships within Apocynaceae (Endress et al., 1996; Civeyrel, 1996; Civeyrel et al., 1998), and this new set of molecular, morpho-palynological, and biomechanical characters should help to resolve the relationships and shifts in reproductive morphology and growth habit outside and inside the subfamily Secamonoideae with other groups of Apocynaceae sensu lato.

Indels have been shown to be useful in phylogenetic reconstruction. Indels in coding regions are generally useful to circumscribe lineages and define evolutionary trends (Hilu & Alice, 1999). In the plastid gene *mat*K, indels occur quite frequently and some are phylogenetically informative (Johnson & Soltis, 1994, 1995; Steele & Vilgalys, 1994; Plunkett et al., 1996, 1997; Xiang et al., 1998; Kron et al., 1999).

Materials and Methods

MATERIALS

Ten sequences belonging to the Secamonoideae are published here for the first time and added to the 46 sequences of Gentianales and Solanales previously published (Civeyrel et al., 1998; Endress et al., 1996). Table 1 provides information on these taxa, their voucher specimens and source, as well as EMBL accession numbers for new sequences. The family Apocynaceae constitutes the ingroup with taxa from Solanaceae, Rubiaceae, Loganiaceae, and Gentianaceae forming the outgroup. In the former Asclepiadaceae, representatives of all subfamilies and tribes were included.

METHODS

DNA preparation. Detailed protocols used have been published by Civeyrel et al. (1998) and will only be summarized here. Total DNA was extracted using the 2X CTAB protocol of Doyle and Doyle (1987). DNA was precipitated using ethanol or propan-2-ol, and proteins were removed with SEVAG (24:1, chloroform and isoamyl alcohol). DNA was purified by ultracentrifugation on a CsCl-ethidium bromide gradient (1.55 g/ml). Double-stranded products of *mat*K were amplified from total DNA

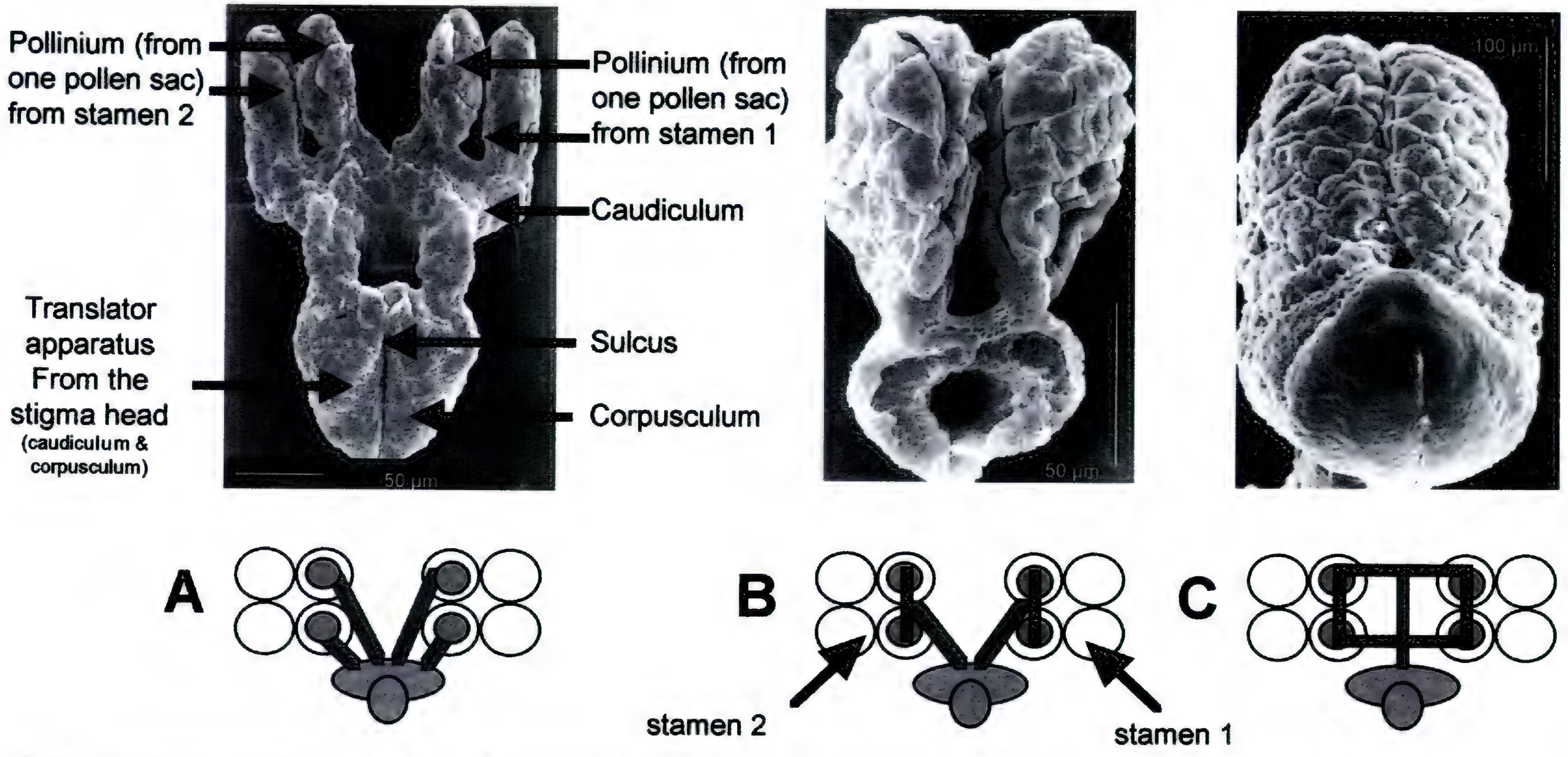


Figure 1. Synorganization within Secamonoideae. —A. *Secamone ligustrifolia*, synorganization only between pollinia and translator apparatus (in gray), no pollinial synorganization. —B. *Secamone dewevrei* subsp. *dewevrei*, intrapollinial synorganization (in black). —C. *Secamone alba*, intra- and interpollinial synorganization (in black).

Table 1. Taxa used in this study. For each taxon, the major subdivisions to which it belongs, geographic area of origin, the voucher for the material used, and EMBL accession numbers used for the 10 newly reported DNA sequences of the plastid gene *mat*K are given; * indicates a sequence published in Endress et al. (1996); ** indicates a sequence published in Civeyrel et al. (1998). Pol indicates when used for pollen. Samples used for biomechanical studies are at the end of the table.

Taxonomic divisions	Genus, species author(s)	Voucher	*Collector*, #	Herbarium	DNA	Pol	EMBL
OUTGROUP							
GELSEMIACEAE							
Gelsemieae	*Gelsemium sempervirens* (L.) J. St.-Hil.	N. Amer.	*Civeyrel 1069*	TL	DNA		*
GENTIANACEAE							
Gentianoideae	*Gentiana verna* L.	Yugoslavia	*Civeyrel 1108*	TL	DNA		*
LOGANIACEAE							
Loganieae	*Geniostoma rupestre* J. R. Forst. & G. Forst.	New Zealand	*Garnock-Jones 2200*	WELTU	DNA		*
Strychneae	*Strychnos nux-vomica* L.	India	*Civeyrel 1096*	TL	DNA		*
PLOCOSPERMATACEAE							
Plocospermeae	*Plocosperma buxifolium* Benth.	Mexico	*Salinas 8050*	Z	DNA		*
RUBIACEAE							
Cinchonoideae							
Cinchoneae	*Cinchona pubescens* Vahl	Peru	*Civeyrel 1063*	TL	DNA		*
Coptosapelteae	*Luculia gratissima* (Wall.) Sweet	India	*Civeyrel 1073*	TL	DNA		*
Ixoroideae							
Gardenieae	*Gardenia thunbergia* L. f.	South Africa	*Civeyrel 1068*	TL	DNA		*
SOLANACEAE							
Solaneae	*Solanum tuberosum* L.	Cultivated	*Du Jardin s.n.*	/	DNA		unpublished
INGROUP							
APOCYNACEAE							
Rauvolfioideae							
Alstonieae	*Alstonia scholaris* (L.) R. Br.	India	FK 212	FTG	DNA		*
Vinceae	*Kopsia fruticosa* (Ker Gawl.) A. DC.	Malaya	*Bremer 3033*	UPS	DNA		*
	Rauvolfia mannii Stapf	Tanzania	*Sennblad 218*	UPS	DNA		*
Tabernaemontaneae	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	India	*Civeyrel 1097*	TL	DNA		*
	Molongum laxum (Benth.) Pichon	Venezuela	*Romero et al. 3016*	Z	DNA		*
Alyxieae	*Chilocarpus suaveolens* Blume	Indonesia	*Chase 1208*	K	DNA		*
Pleiocarpeae	*Picralima nitida* (Stapf) T. Durand & H. Durand	Ivory Coast	*Leeuwenberg 12025*	UPS	DNA		*
Plumerieae	*Plumeria rubra* L.	Nigeria	*Civeyrel 1087*	TL	DNA		*

Table 1. Continued.

Taxonomic divisions	Genus, species author(s)	Voucher	*Collector, #*	Herbarium	DNA	Pol	EMBL
	Thevetia peruviana (Pers.) K. Schum.	Peru	*Civeyrel 1100*	TL	DNA		*
	Allamanda cathartica L.	Brazil	*Civeyrel 1054*	TL	DNA		*
Carisseae	*Acokanthera oblongifolia* (Hochst.) Codd	South Africa	*Civeyrel 1053*	TL	DNA		*
Apocynoideae							
Wrightieae	*Beaumontia grandiflora* Wall.	India	*Civeyrel 1071*	TL	DNA		**
	Nerium oleander L.	France	*Civeyrel 1079*	TL	DNA		**
	Strophanthus divaricatus (Lour.) Hook. & Arn.	China	*Civeyrel 1094*	TL	DNA		*
Apocyneae	*Apocynum androsaemifolium* L.	U.S.A.	*Civeyrel 1058*	TL	DNA		*
Periplocoideae	*Camptocarpus mauritianus* Decne.	Reunion	*Civeyrel 1062*	TL	DNA		**
	Cryptostegia grandiflora R. Br.	Madagascar	*Civeyrel 1221*	TL	DNA		**
	Hemidesmus indicus (L.) R. Br. ex Schult.	India	*Civeyrel 1008*	TL	DNA		**
	Periploca graeca L.	Greece	*Civeyrel 1083*	TL	DNA		**
	Raphionacme welwitschii Schltr. & Rendle	Zaire	*Civeyrel 1088*	TL	DNA		**
	Schlechterella abyssinica (Chiov.) Venter & Verhoeven (ex. *Triodoglossum*)	Ethiopia	*Civeyrel 1102*	TL	DNA		**
Secamonoideae	*Genianthus laurifolius* (Roxb.) Hook. f.	India	*Saldanha & Ramamoorthy 1164*	HIFP		Pol	
	Pervillaea venenata (Baill.) Klack.	Madagascar	*Civeyrel 1248*	TL	DNA	Pol	**
	Pervillaea phillipsonii Klack.	Madagascar	*Civeyrel 1241*	TL	DNA	Pol	AJ312408
	Secamone alba Jum. & H. Perrier	Madagascar	*Humbert & Capuron 2395*	P		Pol	
	Secamone bosseri Klack.	Madagascar	*Civeyrel 1267*	TL	DNA	Pol	**
	Secamone cristata subsp. *densiflora* Klack.	Madagascar	*Civeyrel 1320*	TL	DNA		AJ312400
	Secamone cristata Jum. & H. Perrier	Madagascar	*Decary 15759*	P		Pol	
	Secamone dewevrei De Wild. subsp. *dewevrei*	Zaire	*Becquaert 7052*	K		Pol	
	Secamone buxifolia Decne.	Madagascar	*Civeyrel 1322*	TL	DNA	Pol	AJ312405
	Secamone ecoronata Klack.	Madagascar	*Civeyrel 1261*	TL	DNA		AJ312407
		Madagascar	*Humbert 13408*	P		Pol	
	Secamone elliottii K. Schum.	Madagascar	*Civeyrel 1304*	TL	DNA	Pol	AJ312402
	Secamone falcata Klack.	Madagascar	*Civeyrel 1228*	TL	DNA	Pol	AJ312404
	Secamone geayii Costantin & Gallaud	Madagascar	*Civeyrel 1200*	TL	DNA		**
		Madagascar	*Civeyrel 1241*	TL		Pol	
	Secamone ligustrifolia ssp. *angustifolia* (Decne.) Klack.	Madagascar	*Civeyrel 1324*	TL		Pol	
	Secamone minutifolia Choux	Madagascar	*Civeyrel 1257*	TL	DNA		AJ312406
		Madagascar	*Perrier 16701*	P		Pol	
	Secamone parvifolia (Oliv.) Bullock	Tanzania	*Goyder et al. 3960*	K	DNA		**
	Secamone parvifolia (Oliv.) Bullock	Kenya	*Gillet 13225*	K		Pol	

Table 1. Continued.

Taxonomic divisions	Genus, species author(s)	Voucher	*Collector, #*	Herbarium	DNA	Pol	EMBL
	Secamone sparsiflora Klack.	Madagascar	*Civeyrel 1244*	TL	DNA	Pol	AJ312401
	Secamone uncinata Choux	Madagascar	*Civeyrel 1309*	TL	DNA	Pol	AJ312403
	Secamone volubilis (Lam.) Marais	Reunion	*Civeyrel 1092*	TL	DNA		**
		Reunion	*Bosser 21424*	P		Pol	
	Secamonopsis microphylla Civeyrel & Klack.	Madagascar	*Civeyrel 1206*	TL	DNA	Pol	AJ312409
	Secamonopsis madagascariensis Jum.	Madagascar	*Civeyrel 1262*	TL	DNA	Pol	**
Asclepiadoideae							
Fockeeae	*Fockea capensis* Endl.	South Africa	*Civeyrel 1067*	TL	DNA		**
Marsdenieae	*Dregea sinensis* Hemsl.	China	*Civeyrel 1066*	TL	DNA		**
	Tylophora indica (Burm. f.) Merr.	India	*Civeyrel 1009*	TL	DNA		**
Ceropegieae	*Riocreuxia burchellii* K. Schum.	Africa	*Civeyrel 1109*	TL	DNA		**
Gonolobeae	*Gonolobus xanthotrichus* Brandegee	Mexico	*Civeyrel 1060*	TL	DNA		**
	Matelea quirosii (Standl.) Woodson	Mexico	*Civeyrel 1076*	TL	DNA		**
Asclepiadeae	*Araujia sericifera* Brot.	South Amer.	*Civeyrel 1059*	TL	DNA		**
	Pentarrhinum insipidum E. Mey.	South Africa	*Civeyrel 1081*	TL	DNA		**
	Pergularia daemia (Forssk.) Chiov.	India	*Civeyrel 1000*	TL	DNA		**
	Vincetoxicum nigrum (L.) Moench	France	*Civeyrel 1106*	TL	DNA		**
Samples for biomechanical studies							
	Secamonopsis microphylla Civeyrel & Klack.	Madagascar	*Civeyrel 1386*				
	Secamonopsis madagascariensis Jum.	Madagascar	*Civeyrel 1398*				

Table 2. External and internal primers for *mat*K. The boldfaced letters represent multiple bases present in the primer sequences: K represents the base G T, M represents the base A C and Y represents the base C T.

PRIMER	SEQUENCE
*trn*K 3914F	GGG GTT GCT AAC TCA ACG G
*mat*K -8F	AAT TTC AAA TGG AAG AAA TC
*mat*K 174F	TGT GAA ACG TTT AAT TAA TC
*mat*K 174R	CGA **K**TA ATT AA**M** CGT TTC AC
*mat*K 503F	TCG CTA TTG GGT AAA AGA TGC
*mat*K 503R	GCA TCT TTT ACC CAA TAG CG
*mat*K 681F	GTG AAT ACG AAT C**Y**A TTT TC
*mat*K 900F	TGG AAA TTT TAC CTT GTC AA
*mat*K 1309F	GAC TTT CTT GTG CTA GAA CT
*mat*K 1628R	CAT GCT ACA TCA ACA TTT CAG
*trn*K -2R	AAC TAG TCG GAT GGA GTA G

using one of the *trn*K primers in combination with an internal primer (list given in Table 2). Direct sequencing of the double-stranded PCR products was performed using the Taq Dye Deoxy™ Terminator Cycle Sequencing Kit. Excess dye terminators were removed using Centri-sep spin columns. Direct sequencing was performed on an ABI 373A DNA sequencer, and sequences were edited using the programs Sequence Navigator™ and Auto-Assembler™ (Applied Biosystems, Warrington, Cheshire).

ANALYSIS

For this study all but an average of the first 50 bases at the 5′ end of *mat*K were sequenced (our primers were not located so that we could accurately determine the sequence near the forward PCR primer). In all cases, sequences were aligned visually against the published *Solanum tuberosum* L. sequence (EMBL-Z11741, Du Jardin, unpublished) (aligned matrix available from author upon request). Alignment was straightforward; the length of the individual *mat*K sequences varied between 1509 and 1551 bp, and there were 24 insertions and deletions (indels), which are in triplets (involving 3 to 21 bp). Indels often consisted of the repetition of a sequence of base pairs present just before the indel itself. Seven of these indels are phylogenetically informative, and two are homoplasious (occurring in two distant genera). None of the indels were coded in the analysis, but inserted regions were retained and coded as missing. In total, the matrix was 1653 bp long with 443 potentially parsimony-informative characters (27%).

Cladistic analysis was performed using PAUP 3.1.1. (Swofford, 1993) with the following options: heuristic search, 1000 replicates of random taxon-additions, and TBR swapping. Two separate phases were performed: the first one with 1000 replicates, with complete swapping on all trees accumulated in the replicates (which should have found all trees at that length); the second, successive approximations weighting (hereafter SW; Farris, 1969), with characters reweighted according to their rescaled consistency index (RC) based on the best fit of characters on any of the trees. Reasons for using SW have been explained in a previous paper (Civeyrel et al., 1998). Rounds of re-weighting were repeated until the tree length did not change in two consecutive iterations. Confidence in specific clades of the resulting topology was estimated by bootstrap analysis. The following settings were used: 1000 replicates, keeping bootstrap frequencies from 1 to 100%, which were compatible with the 50% majority rule consensus tree, simple addition of taxa, sampling characters with equal probability but applying weights (from SW), and NNI swapping (nearest-neighbor interchange) but holding only 25 trees per step. All illustrated trees use the ACCTRAN optimization. The base weight of 1000 applied in SW was removed for tree presentation.

POLLINARIUM PREPARATION

Samples examined are from the herbarium collection at the Royal Botanical Gardens, Kew, from the author's alcohol collection, and from fieldwork in Madagascar. Pollinaria were removed from flowers under a dissecting microscope, transferred to 100% ethanol, air dried on stubs, and coated with platinum using a Balzers Sputter Coater SPD 050. Entire flowers were prepared for observation by critical-point drying in a Balzers CPD 030; tissues were dehydrated in a graded ethanol series, transferred to acetone and to the CPD, and then observed with a Hitachi S2400 scanning electron microscope (SEM).

BIOMECHANICS AND ANATOMY

Measurements of flexural stiffness, *EI* (Newtons times square meters: Nm^2), structural Young's modulus, *E* (Mega Newtons per square meter: MNm^{-2}), and axial second moment of area, *I* (mm^4), of *Secamonopsis microphylla* and *S. madagascariensis* were taken during fieldwork in Madagascar in April 1999 near Tulear (23°24′S, 43°47′E). Flexural stiffness represents the tangible resistance to bending of a structure and is the product of the structural Young's modulus and the axial second moment of area. Structural Young's modulus is a value that

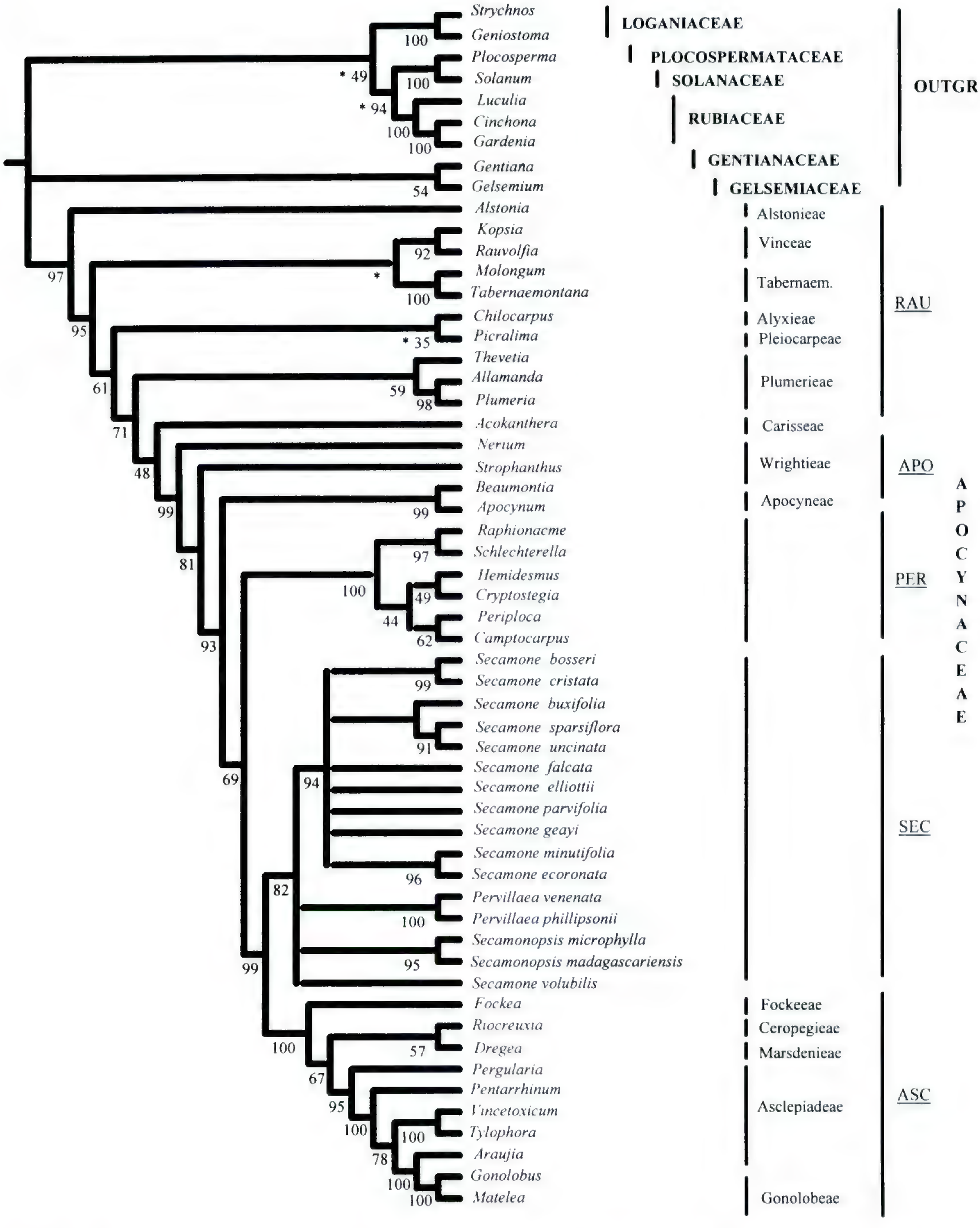


Figure 2. Strict consensus tree of the 25 most parsimonious trees obtained with the successive weighting analysis. Bootstrap values are shown below the branch. Abbreviations: APO, Apocynoideae; RAU, Rauvolfioideae; PER, Periplocoideae; SEC, Secamonoideae; and ASC, Asclepiadoideae; * denotes branches not present in the unit weighted strict consensus tree. This follows the classification published by Endress and Bruyns (2000).

describes the elastic mechanical properties of materials and is currently used for describing and comparing quantitatively changes in mechanical properties of plant stems during ontogeny (Rowe & Speck, 1998; Speck & Rowe, 1999): compliant or flexible materials have low Young's moduli, whereas stiffer materials have higher moduli. Stem segments from basal to distal parts of plants were pruned from living plants and submitted to mechanical bending tests within several hours of being cut.

	Indel A	Indel C
		111111111111
	11111111111111111111111111	111111111222
	00000011111111112222222223	999999999000
Taxon	45678901234567890123456890	234567890123
Riocreuxia burchellii	CAT------AGTTTAAA**TTTAAAC**CGA	ATTAG**G**AATAAG
Pentarrhinum insipidum	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Dregea sinensis	CAT------AGTTTAAAC------CGA	ATTAG**G**AATAAG
Pergularia daemia	CAT------AGTTTAAAC------CGA	ATT------AAG
Vincetoxicum nigrum	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Tylophora indica	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Araujia sericifera	C-T------AGTTTAAAC------CGA	ATTAGCAATAAG
Gonolobus xanthotrichus	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Matelea quirosii	CAT------AGTTTAAAC------CGA	ATTAGCAATAAG
Fockea capensis	CAT------AGTTTAAAC------CGA	ATTAG**G**AATAAG
Secamone bosseri	CAT--------------------CGA	ATT------AAG
S. cristata ssp. densiflora	CAT--------------------CGA	ATT------AAG
S. sparsiflora	CAT--------------------CGA	ATT------AAG
S. uncinata	CAT--------------------CGA	ATT------AAG
S. elliottii	CAT--------------------CGA	ATT------AAG
S. geayi	CAT--------------------CGA	ATT------AAG
S. falcata	CAT--------------------CGA	ATT------AAG
S. buxifolia	CAT--------------------CGA	ATT------AAG
S. minutifolia	CAT--------------------CGA	ATT------AAG
S. ecoronata	CAT--------------------CGA	ATT------AAG
S. parvifolia	CAT--------------------CGA	ATT------AAG
S. volubilis	CATGACCATGGTTTAAAC------CGA	ATT------AAG
Pervillaea venenata	CAT------AGTTTAAAC------CGA	ATT------AAG
P. phillipsonii	CAT------AGTTTAAAC------CGA	ATT------AAG
Secamonopsis microphylla	CAT------GGTTTAAAC------CGA	ATT------AAG
Se. madagascariensis	CAT------GGTTTAAAC------CGA	ATT------AAG
Hemidesmus indicus	CAT------AGTTTAAAC------CGA	ATT------AAG
Raphionacme welwitschii	CAT------AGTTTAAAC------CGA	ATT------AAG
Schlechterella abyssinica	CAT------AGTTTAAAC------CGA	ATT------AAG
Cryptostegia grandiflora	CAT------AGTTTAAAC------CGA	ATT------AAG
Periploca graeca	CAT------AGTTTAAAC------CGA	ATT------AAG
Camptocarpus mauritianus	CAT------AGTTTAAAC------CGA	ATT------AAG
Allamanda cathartica	CAT------AGTTTAAAC------CGA	ATT------AAG
Nerium oleander	CAT------AGTTTAAAC------CGA	ATT------AAG
Apocynum androsaemifolium	CAT------AATTTAAAC------CGA	ATT------AAT
Beaumontia grandiflora	AAT------AGTTTAAAC------CGA	ATT------AAG
Strophanthus divaricatus	CAT------AGTTTAAAC------CGA	ATT------AAG
Acokanthera oblongifolia	CAT------AGTTTAAAC------CGA	ATT------AAG
Alstonia scholaris	CAT------AGTTTAAAC------CGA	ATT------AAG
Chilocarpus suaveolens	CAT------AGTTTAAAC------CGA	ATT------AAG
Kopsia fruticosa	CAG------AGTTTAAAC------CGA	ATT------AAG
Molongum laxum	CAT------AGTTTAAAC------CGA	ATT------AAG
Picralima nitida	CAT------AGTTTAAAC------CGA	ATT------AAG
Plumeria rubra	CAT------AGTTTAAAC------CGA	ATT------AAG
Rauvolfia mannii	CTA----------AAC------CGG	ATT------AAG
Tabernaemontana divaricata	CAT------AGTTTAAAC------CGA	ATT------AAG
Thevetia peruviana	CAT------AGTTTAAAC------CGA	ATT------AAG
Gentianales outgroups	***------*********------***	***------***
Solanum tuberosum	CAT------GGTTTAAATAG**AAATAG**G	***------***

Figure 3. Aligned sequences of the plastid gene *mat*K showing the three main phylogenetically informative indels A, B, and C. Numbers indicate position of nucleotides that are numbered consecutively from 5′ to 3′, dashes indicate gaps, question marks an unresolved sequence as for *Allamanda cathartica*, and bold sequences repeat underlined sequences. The sequences were replaced by a line of asterisks (**) for the outgroup taxa where they were present.

```
                                  Indel B

                                  6666666666666666666666666666666666666666666666666666
                                  4444455555555556666666666777777777888888888999999999
Taxon                             5678901234567890123456789012345678901234567890123456789
-------------------------------------------------------------------------------------------
Riocreuxia burchellii             AAAGAAAACCAGAAAGAAAACCAGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Pentarrhinum insipidum            AAAGAAAACT----------------------TTTTTAACAAAAGGAACTCTAAAA
Dregea sinensis                   AAAGAAAACCAGAAAGAAAACCAGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Pergularia daemia                 AAAGGAAATC------------GGTTTTCATTTTTTAACAAAACGAAATCTAAAA
Vincetoxicum nigrum               AAAGAAAACC------------AGTTTTCATTTTTTAACAAAAAGAAATCTAAAA
Tylophora indica                  AAAGAAAACC------------AGTTTTCATTTTTTAACAAAAAGAAATCTAAAA
Araujia sericifera                AAAGAAAACC------------AGTTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Gonolobus xanthotrichus           AAAGAAATCC------------AGCTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Matelea quirosii                  AAAGAAATCC------------AGTTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Fockea capensis                   AAAGAAAACT------------TGTTTTCTTTTTTTAACAAAAAGAAATCTAAAA
Secamone bosseri                  AAAGAAAACC------------AGTTTTCATTTTTTGACA------------AAA
S. cristata ssp. densiflora       AAAGAAAACC------------AGTTTTCATTTTTTTAACA------------AAA
S. sparsiflora                    AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. uncinata                       AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. elliottii                      AAAGAA---C------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. geayi                          AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. falcata                        AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. buxifolia                      AAAGAAAACC------------AGTTTTCATTTTTTTA---AAAAGAAATCTAAAA
S. minutifolia                    AAAGAA---C------------TGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. ecoronata                      AAAGAAAACC------------TGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
S. parvifolia                     AAAGAAAACC------------AGTTTTCGTTTTTTTAACAAAAAGAAATCTAAAA
S. volubilis                      AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Pervillaea venenata               AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
P. phillipsonii                   AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Secamonopsis microphylla          AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Se. madagascariensis              AAAGAAAACC------------AGTTTTCATTTTTTTAACAAAAAGAAATCTAAAA
Hemidesmus indicus                AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATCCAAAA
Raphionacme welwitschii           AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATCCAAAA
Schlechterella abyssinica         AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATCCAAAA
Cryptostegia grandiflora          AAAGAAAACC------------AGGTTTCATTTTTTTAACAAAAAGAAATTCAAAA
Periploca graeca                  AAAGAAAACC------------AGGTTTCGTTTTTTTAACA------------AAA
Camptocarpus mauritianus          AAAGAAAACC------------AGGTTTCATTTTTTTAACA------------AAA
Allamanda cathartica              ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Nerium oleander                   AAARAAAACC------------AGKTTTCATTTTTTTAACA------------AAA
Apocynum androsaemifolium         AAAGAAACCC------------GGTTTTCTTTTTTTAACA------------AAA
Beaumontia grandiflora            AAAGAAARCC------------AGTTTTCATTTTTTTAACAAAAAGAAATYCAAAA
Strophanthus divaricatus          AAAGAAAACC------------AGTTTCAATTTTTTTAACA------------AAA
Acokanthera oblongifolia          AAAGAAACCC------------AGTTTTTATTTTTTTAACA------------AAA
Alstonia scholaris                AAAGAAACCC------------AGTTTTTCTTTTTTTAACA------------AAA
Chilocarpus suaveolens            AAAGAAACCC------------GGTTTTGATTTTTTTAACA------------AAA
Kopsia fruticosa                  AAAAAAACCC------------AGTTTCGATTTTTTTAACA------------AAA
Molongum laxum                    AAAGAAAGAC------------AGTTTTTATTTTTTTAACA------------AAA
Picralima nitida                  AAAGAAAGCC------------GGTTTTGCTTTTTTCACA------------AAA
Plumeria rubra                    AAAGAAACCC------------AGTTTGGATTTTTTTAACA------------AAA
Rauvolfia mannii                  AAAGAACCCC------------AGTTTTTCTTTTTTTAACA------------AAA
Tabernaemontana divaricata        AAAGAAAGTC------------GGTTTTGATTTTTTTAACAAAAATAAATCCAAAA
Thevetia peruviana                AAAGAACCCC------------CGCTTGGATTTTTTTAACA------------AAA

Gentianales outgroups             **********----------**********----------AAA
Solanum tuberosum                 **********----------**********----------***
```

Figure 3. Continued.

Representative axis segments were selected from the entire plant in order to observe trends in mechanical properties all over the stem system for both shrub and liana. The bending tests were carried out on a steel frame bending apparatus in the close vicinity of the collected sample. Span distance of the tested stems and weight increments were varied according to the size and bending resistance of the material selected (see Rowe & Speck, 1996; Speck & Rowe, 1999). Segments of each tested stem were retained in FAA for anatomical and developmental analyses of plant axes.

Results

PHYLOGENETIC ANALYSIS

The unit weight analysis of the complete data set resulted in 600 trees 1679 steps long, with a consistency index of CI = 0.642 (0.530 excluding uninformative characters) and a retention index of RI = 0.730. The successive weighting analysis resulted in 25 trees 1407 steps long (base weight of 1000 applied in Successive Weighting, SW, removed), with a consistency index of CI = 0.867 (0.725 excluding uninformative characters) and a retention index of RI = 0.900. The strict consensus tree of the unit weighting analysis and from the successive weighting are almost identical; only four branches at the base of the tree are not present in the unit weighting analysis. Therefore, comments in the discussion will be based only on the strict consensus tree (Fig. 2) of the SW analysis. In this analysis three indels, occurring in several taxa (Fig. 3), have been examined as putative molecular markers for this phylogeny. They are usually repetitions (represented in bold) of an adjacent sequence (with this adjacent sequence underlined) (Fig. 3).

POLLINARIUM STRUCTURE AND INSERTION

The morphology of non-acetolyzed pollinaria of Secamonoideae was examined and morphological differences were observed in pollinia, corpusculum, and caudicula (Civeyrel, 1994, 1996), as well as pollinia insertion. The corpusculum is coffee bean-shaped, with a more or less narrow slit facing the pollinator. The back of the corpusculum is more or less spongy with perforations of different sizes; the front is generally more compact with a verrucate or smooth surface. There are one or two caudicula bearing the pollinia; when there is only one caudiculum, it can be hemispherical (Fig. 4A, B, G) or elongated (Fig. 4D, C, E) and with the pollinia almost sessile on the back. In some cases the corpusculum is surrounded by a caudiculum without a distinct shape bearing four fused pollinia (Figs. 4F, H, I, 5A, B, D, E). When two caudicula are present, they are either short (Figs. 1B, 5F) or long (Fig. 5C, G), and bear a pair of fused pollinia. The samples examined included pollinia arranged in all possible configurations of synorganization. They may be disposed all around the caudiculum (Fig. 4A, B, C, G) or tiered in pairs when there is no pollinial synorganization (Fig. 4D, E). They are fused in pairs when there is intrapollinial synorganization (Figs. 1B, 5C, F, G) or in a unit of four in the case of intra- and interpollinial synorganization (Figs. 4F, H, I, 5A, B, D, E). A reduction of pollen walls between fused pollinia has also been observed (Civeyrel, 1995).

Pollinium insertions have been examined in *Secamone geayi* and in *S. buxifolia*. In *Secamone buxifolia* only a part of the pollinarium was inserted into the pollination chamber, and sometimes only one pollinium, whereas in *Secamone geayi* the entire pollinarium was inserted.

BIOMECHANICAL RESULTS OF A SECAMONOID SHRUB AND LIANA

Both *Secamonopsis madagascariensis*, a twining liana, and *S. microphylla*, a shrub, show reductions in Young's modulus of the stem during ontogeny. Plant ontogeny is depicted here as increasing stem diameter as indicated by the increase in *I*, second moment of area, in Figure 6. Young distal stages of *S. microphylla* show relatively high values of *E* for the stem of just over 5100 MNm^{-2}; this value drops during ontogeny to around 2000 MNm^{-2}. The drop in Young's modulus during ontogeny is more marked in the twining liana *S. madagascariensis*; young distal stages show values of just over 2000 MNm^{-2} and this is followed by a drop to as low as just over 300 MNm^{-2} in the oldest sample measured.

Discussion

STRICT CONSENSUS TREE

The three subfamilies of the former Asclepiadaceae are monophyletic, with Secamonoideae as sister group of the Asclepiadoideae (Fig. 2). Asclepiadoideae and Periplocoideae are both strongly supported, each with a bootstrap value of 100%; the Secamonoideae are supported by a bootstrap value of 82%. The composite group formed by the three subfamilies of the former Asclepiadaceae is less well supported with a bootstrap value of only 69%. The monophyly of the former Asclepiadaceae, which is only poorly supported here, has been much questioned in recent years (Sennblad & Bremer, 1996; Sennblad et al., 1998; Sennblad & Bremer, 2000; Potgieter & Albert, 2001 this volume) and is far from being resolved. The systematic position of the Periplocoideae, however, is beyond the scope of this paper. Here we will focus on the relationships within Secamonoideae.

Within Secamonoideae five groups are strongly supported by bootstrap values above 90% (Fig. 2). These groups are: (1) *Secamonopsis*, (2) *Pervillaea*, (3) the two species belonging to the *Secamone cristata* group sampled here (*S. cristata* subsp. *densiflora* and *S. bosseri*), and two groups

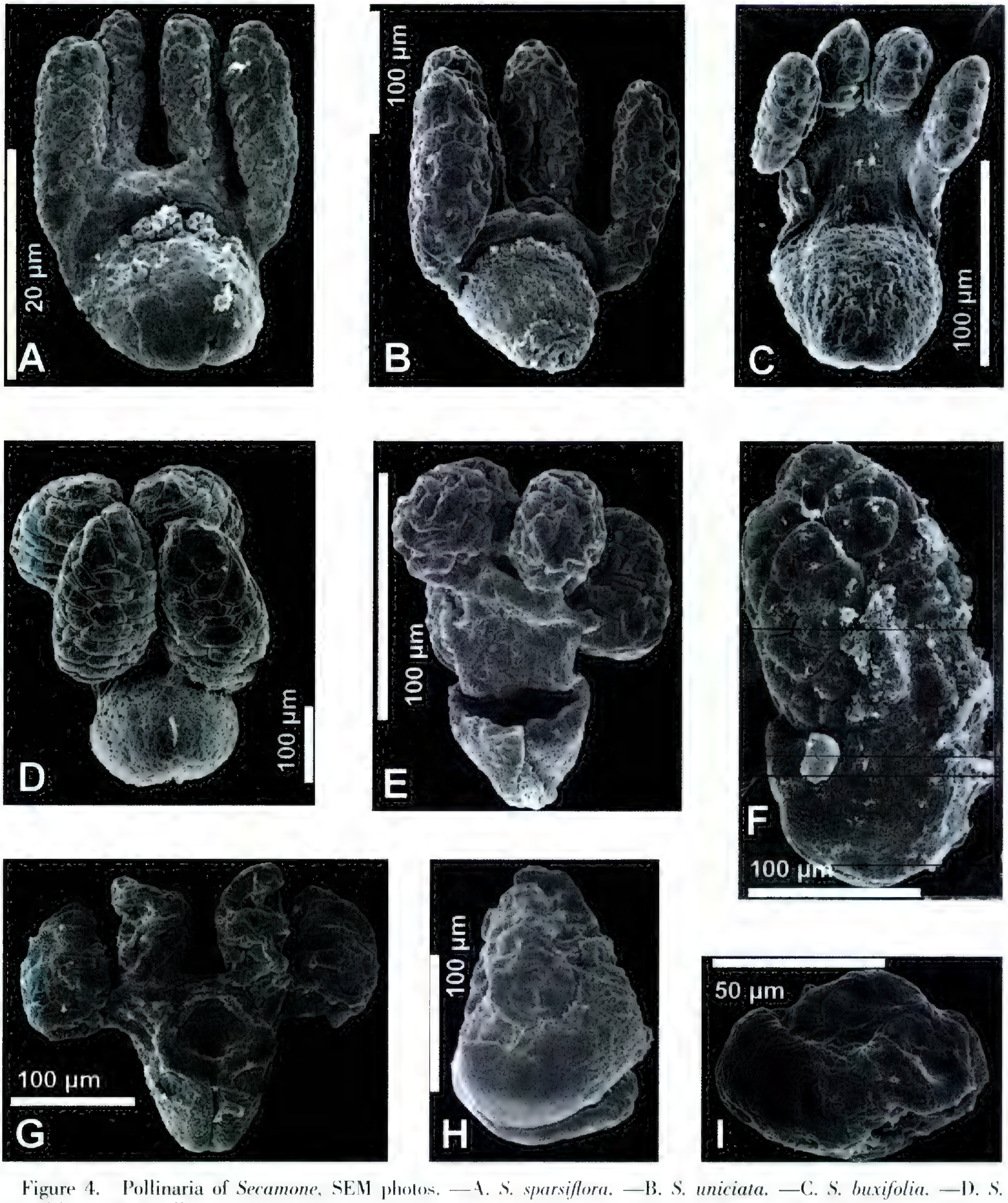
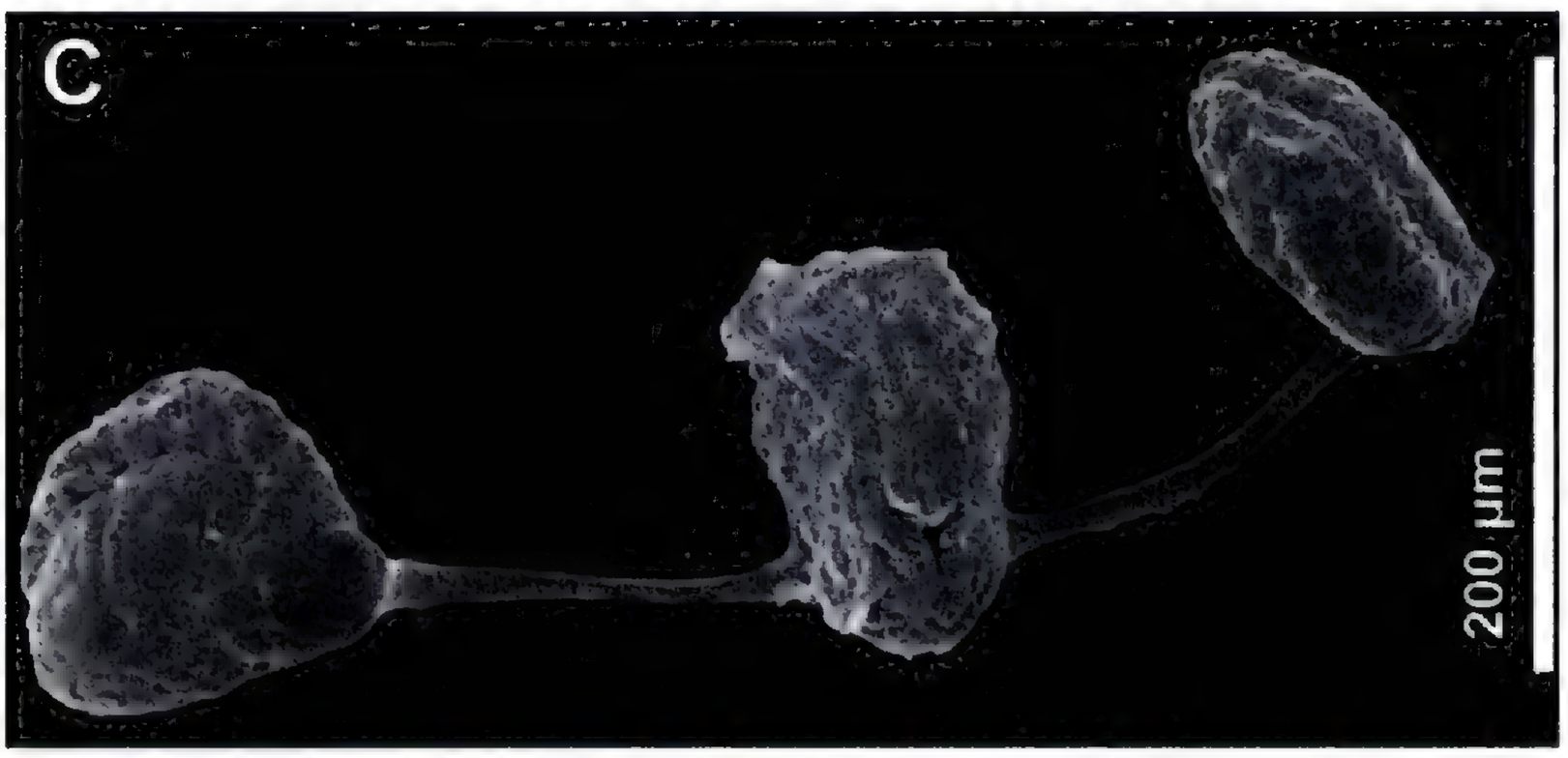


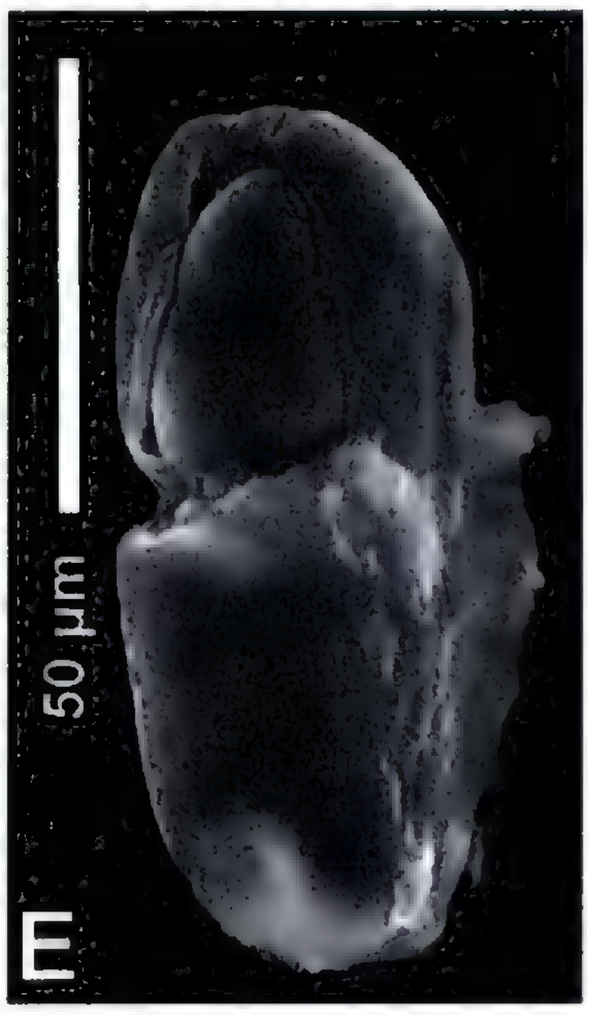
Figure 4. Pollinaria of *Secamone*, SEM photos. —A. *S. sparsiflora*. —B. *S. uniciata*. —C. *S. buxifolia*. —D. *S. parvifolia*. —E. *S. elliottii*. —F. *S. geayi*. —G. *S. falcata*. —H. *S. ecoronata*. —I. *S. minutiflora*. Voucher specimens are cited in Table 1.

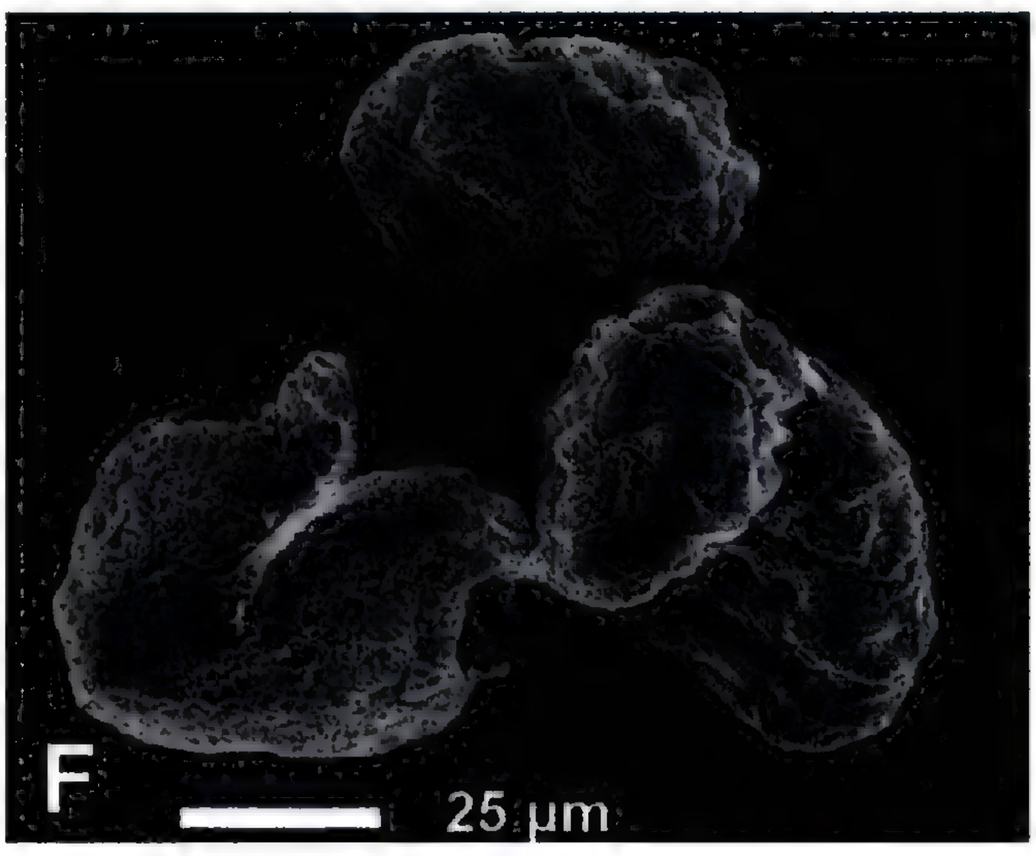
within the remainder of the *Secamone* clade, (4) *S. sparsiflora* and *S. unciata*, and (5) *S. minutifolia* and *S. ecoronata*. There is no strong support for the position of *Secamone volubilis*, endemic to Reunion Island. The only African *Secamone* included in this study, *S. parviflora*, is associated with the poorly resolved clade of Malagasy *Secamone*. Unless more sampling is done on species from the African mainland and from Asia and Australasia it will be difficult to assess the origin of the Madagascan species.

INDELS

A six-base insertion from bp 1195 to 1200 characterizes the Asclepiadoideae group (with the no-







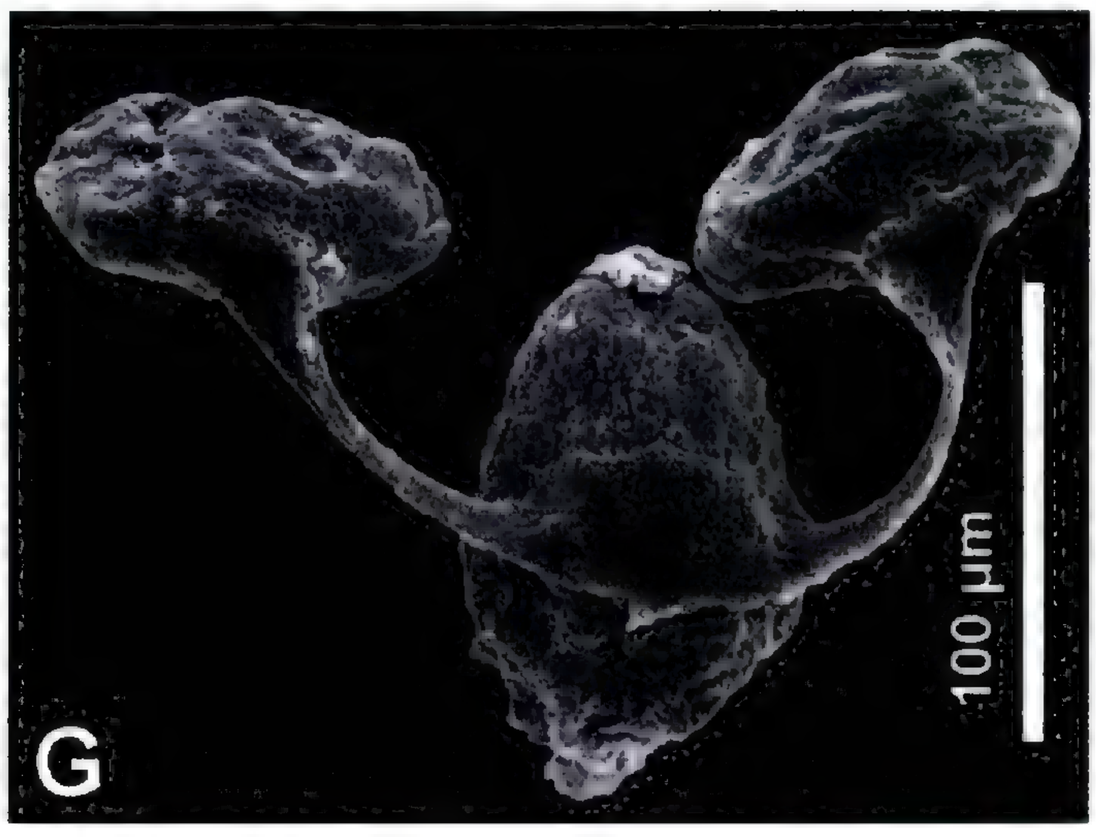


Figure 5. Pollinaria of Secamonoideae, SEM photos. —A. *Pervillea venenata.* —B. *Pervillea phillipsonii.* —C. *Secamonopsis madagascariensis.* —D. *Secamone bosseri.* —E. *Secamone cristata.* —F. *Genianthus laurifolius.* —G. *Secamonopsis microphylla.* Voucher specimens are cited in Table 1.

table exception of *Pergularia daemia*), and has not been found in any other Apocynaceae or among the outgroup taxa (Fig. 3, C region). Within this six-pair base insertion there is only one change: in the basal taxa *Fockea*, *Riocreuxia*, and *Dregea*, a G (2′-deoxyguanosine) is found on position 1197, replacing the C (2′-deoxycytidine) found in all other taxa. This insertion could be useful for identifying possible members of the Asclepiadoideae, such as sterile herbarium specimens within the family Apocynaceae, which contains between 4000 and 6000 species.

Another interesting indel that we have been investigating is actually a series of indels found in the *mat*K region from 639 to 696 bp (Fig. 3, B region). It has been found in all the former Asclepiadaceae, with the exception of two taxa belonging to the *Secamone cristata* group and two species of Periplocoideae in *Periploca* and *Camptocarpus*. This insertion has also been found in two unrelated taxa in the basal Apocynaceae: *Beaumontia* and *Tabernaemontana*. Looking closely at the base composition of this insertion, it can be seen that in all Periplocoideae and in *Beaumontia* and *Tabernaemontana*, there is a C at position 695, whereas there is a T (2′-deoxythymidine) for all Secamonoideae and Asclepiadoideae. In this same region two small indels (bp 651–653) have been found in two species of the genus *Secamone*: *S. elliottii* and *S. minutifolia*. There are also two insertions (bp 656–666) in two taxa belonging to different tribes of the Asclepiadoideae in *Riocreuxia* (Ceropegieae) and *Dregea* (Marsdenieae).

The third indel, and probably the most interesting one for our study, is a nine bp deletion (bp 118–126) that has only been found in the Madagascan *Secamone* clade including the African species, but not in *Secamone volubilis* from the Mascarenes.

SYNORGANIZATION

The type of pollinium synorganization between the contents of different pollen sacs we have described in Secamonoideae is not known to occur anywhere else in the angiosperms. A comparison of the geographic distribution of this character has shown that intrapollinial synorganization is much more widespread than interpollinial synorganization. Intrastaminal synorganization is found in taxa in both Asia and Africa and Madagascar, and occurs in *Secamone*, *Genianthus*, *Pervillaea*, *Secamonopsis* (Civeyrel, 1996), *Toxocarpus*, and *Calyptranthera*. In general this feature is linked with two caudicula such as in the pollinaria of *Secamone dewevrei* (Fig. 1B), *Secamonopsis madagascariensis* and *S. microphylla* (Fig. 5C, G), or *Genianthus laurifolius* (Fig. 5F). Interpollinial synorganization has a much narrower distribution; it has been found only in Madagascar and does not extend beyond this island, not even in the Mascarenes or the Comoro Islands. It occurs in three different genera: *Pervillaea* (Civeyrel, 1996; Klackenberg, 1996b) and *Calyptranthera* (see Klackenberg, 1996a, 1997, 1998, for illustration of pollinarium), both endemic to Madagascar, and in some species of the widely distributed genus *Secamone* (Civeyrel, 1994). Unfortunately, we do not have sequences for all species of *Pervillaea*, and those of *Calyptranthera* are yet to be sequenced. Klackenberg (1996b), however, has suggested that *Calyptranthera* is the sister group of the genus *Pervillaea*, where all the examined species have interstaminal synorganization. We have mapped the distribution of this character onto our molecular tree (Fig. 7A) to estimate character evolution among species in Madagascar. Based on the phylogeny presented here, pollinial synorganization has evolved twice within this group, since *Pervillaea* lies outside of the group of *Secamone*. It can also be seen that only one type of pollinial synorganization occurs for each of our well supported groups within Secamonoideae. This has to be confirmed by more sampling within the genus *Secamone*, however.

One way to view the evolution of pollinial synorganization is to relate it to pollination and pollinium insertion. We have started to examine pollination in different species exhibiting different degrees of synorganization. For species without pollinial synorganization such as in the Madagascan species *Secamone buxifolia* (Fig. 4C), we have noted that when there is an insertion by an insect, only one pollinium is inserted, either alone, or sometimes still attached to the translator apparatus. Kunze (1991) has also demonstrated this with *Secamone alpinii*. The reverse occurs in species such as *Secamone geayi* (Fig. 4F), another Madagascan species, with interpollinial synorganization, where we have seen that the entire pollinarium (i.e., all four pollinia and the translator) is inserted. Fused pollinia with intrapollinial synorganization are very strongly glued together, and in *Secamone*, for example, it is almost impossible to separate the pollinia from the corpusculum without breaking them. The fusion observed may reduce the risk of losing pollinia during transport. But since all four pollinia are fused into a unit, it also means that such a unit can only be distributed once. Conversely, free pollinia (i.e., with neither intrapollinial nor interpollinial synorganization) can be distributed among up to four different flowers. When no pollinial synor-

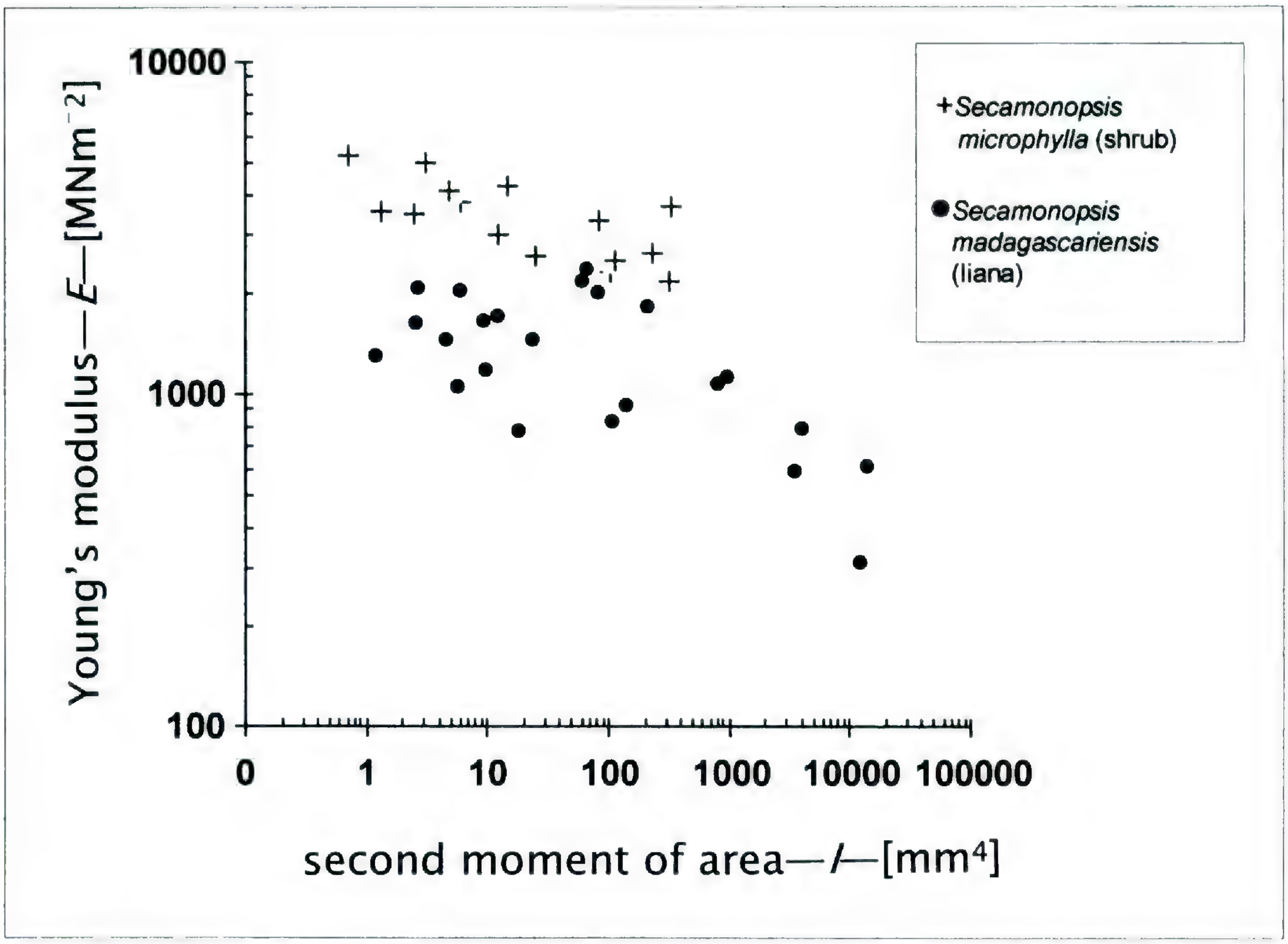


Figure 6. Double logarithmic plot of biomechanical data for two species of *Secamonopsis*. Structural Young's modulus (E) is plotted against axial second moment of area (I) of stems sampled throughout the plant body. Both the shrub (*S. microphylla*) and liana (*S. madagascariensis*) show a decrease in E during ontogeny which is characteristic of woody lianas (Speck & Rowe, 1999).

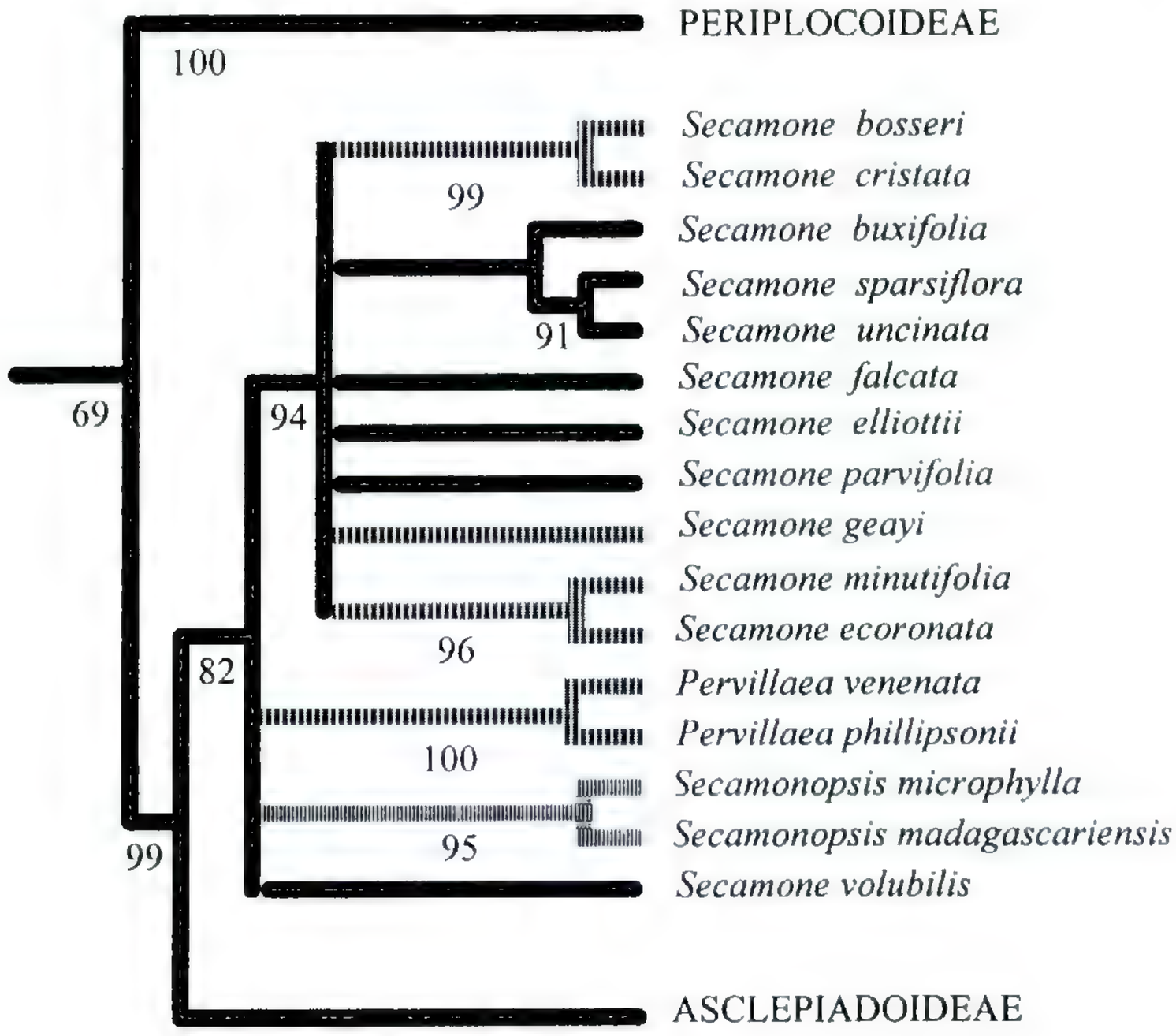
ganization is present, however, the risk of losing pollinia during transport may be high, as pollinia are sometimes only loosely attached to the translator apparatus (Civeyrel, 1996) and can easily fall from it (Friedmann, 1990). Pollination success for flowers with intrapollinial synorganization might be intermediate between these two extremes in terms of pollinia loss and numbers of flowers pollinated, and this would be interesting to investigate.

BIOMECHANICS

In addition to studying reproductive traits during the evolution of this group, we have begun to incorporate biomechanical studies for investigating evolution of growth forms. Distribution of plant growth forms mapped onto the molecular tree suggests that self-supporting shrubs have evolved from lianescent forms at least five times within the Secamonoideae for the examples investigated herein (Fig. 7B). In the genus *Secamonopsis*, *S. madagascariensis* is a twining liana with stiff searchers (young stems) and flexible basal stages; *Secamonopsis microphylla* is a small semi-erect shrub. The phylogeny suggests that the "self-supporting" growth form here is derived within the lianescent group. As expected, the liana species shows a typical drop in the value of E (Young's elastic modulus) for the stem during ontogeny as has been documented for a variety of woody lianas (Rowe & Speck, 1996; Speck & Rowe, 1999). What is surprising here is that the shrub actually shows a drop in stem elastic modulus during ontogeny as well. This appears to explain the remarkable habit of this shrub, in which older branches are semi-recumbent

→

Figure 7. —A. Pollinial synorganization. —B. Biomechanical aspects. Characters mapped onto the strict SW analysis consensus tree of the 25 most parsimonious trees obtained with the successive weighting analysis. Bootstrap values are shown above the branches.

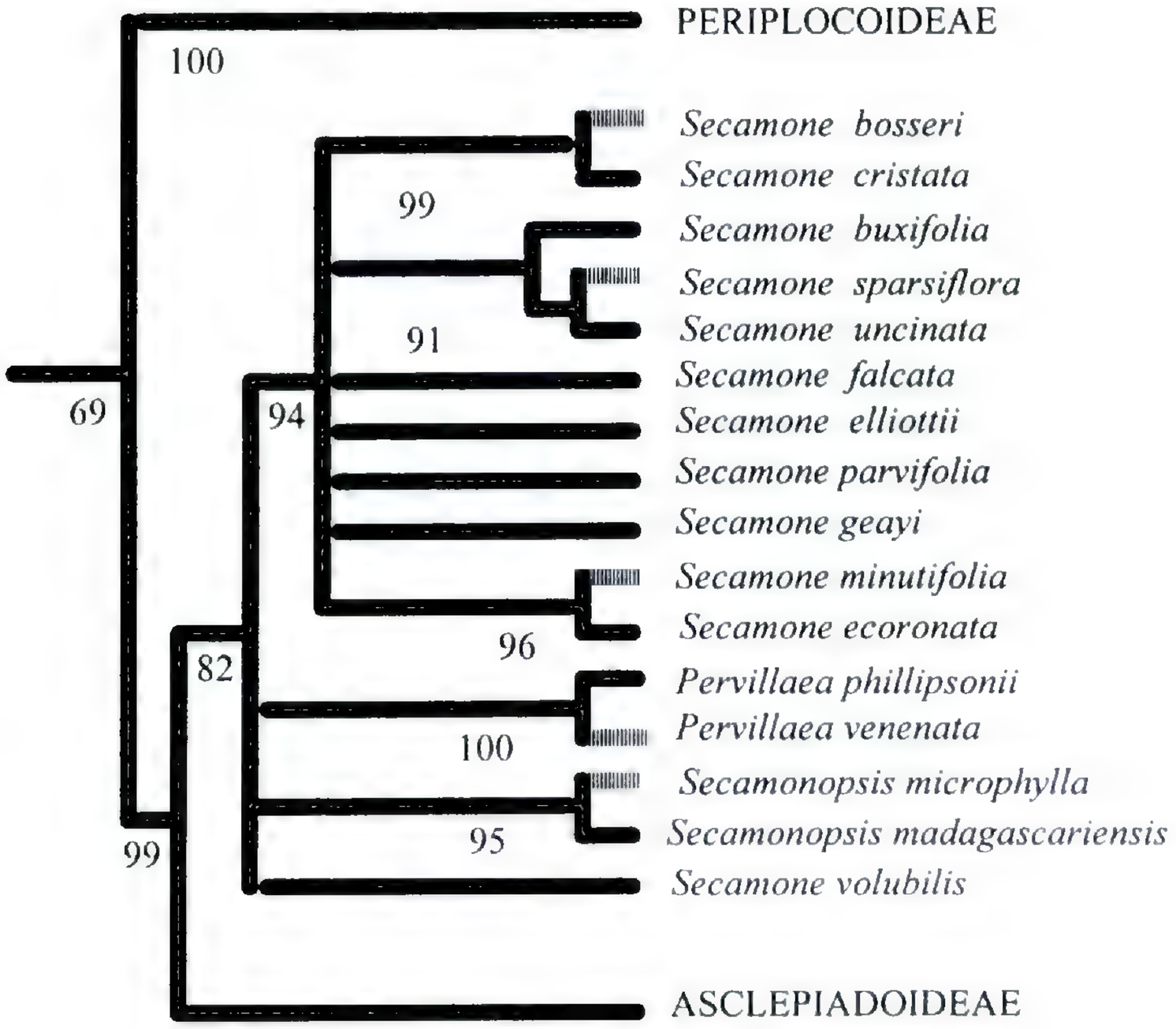


A

No pollinial synorganization

intrapollinial synorganization

interpollinial synorganization



B

Twining liana

Self supporting shrub

and lean against each other or along the ground with the more rigid younger branches oriented vertically. Interestingly, the values of *E* for the shrub are higher than the liana, and this may also reflect the difference between the mechanics of the shrub-like form and the liana. Initial observations of the anatomy of the two plants indicate that the shrub has a much denser wood, fewer and smaller vessels, and a relatively narrow band of compliant outer secondary phloem and bark compared with the liana. Ongoing investigations will quantify the contribution of each tissue to the mechanics of the stem during ontogeny; it will then be possible to determine more exactly which developmental traits cause the mechanical patterns observed and thus explain, for example, why the "shrub-like" plant has retained a basically lianescent mechanical signal. Further investigations will also sample additional genera from the Secamonoideae, particularly in terms of assessing shrub-like or self-supporting habits derived from within a largely lianescent clade. Plant growth forms have been common characters in phylogenetic analyses with character states assigned to trees or shrubs or herbs, and so on. However, growth forms themselves are clearly complex aspects of a plant's life history and are the result of a complex array of developmental traits. We hope that both biomechanical and anatomical approaches combined with phylogenetic techniques as outlined here may provide a means of determining the underlying developmental processes in the evolution of different growth forms.

The Secamonoideae have retained some ancestral characters such as four pollinia per stamen and a relatively simple translator apparatus in which the pollinia are only weakly attached to the translator, which help us to understand the evolution of the reproductive system of the entire family Apocynaceae. The Secamonoideae have also evolved some unique characters among angiosperms such as pollinial synorganization, which links together pollen from different pollen sacs and anthers. With their distribution of many endemic taxa, and their remarkable speciation in Madagascar, a detailed phylogenetic study of the Secamonoideae also enables us to study some fundamental aspects of plant evolution, such as changes in reproduction and overall growth form.

Literature Cited

Brown, R. 1810. On the Asclepiadeae. London. Preprint of Brown, R. 1811. On the Asclepiadeae, a natural order of plants separated from the Apocyneae of Jussieu. Mem. Wern. Nat. Hist. Soc. 1: 12–78.

Bruyns, P. V. & P. I. Forster. 1991. Recircumscription of the Stapelieae (Asclepiadaceae). Taxon 40: 381–391.

Civeyrel, L. 1994. Variation and evolution of pollen types in the genus *Secamone* (Asclepiadaceae, Secamonoideae). Compt. Rend. Acad. Sci. Paris 317: 1159–1165.

———. 1995. Pollen morphology and ultrastructure of the genus *Secamone* in Africa. Pp. 207–215 *in* A. Le Thomas & E. Roche (editors), 2nd Symposium on African Palynology, Tevuren, Belgium. Publ. Occas. CIFEG 31, Orleans.

———. 1996. Phylogénie des Asclepiadaceae, Approche Palynologique et Moléculaire. Ph.D. Thesis, Université Montpellier II. [Unpublished.]

——— & J. Klackenberg. 1996. A second species of the genus *Secamonopsis* (Asclepiadaceae). Novon 6: 144–146.

———, A. Le Thomas, I. K. Ferguson & M. W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *mat*K sequences. Molec. Phylogenet. Evol. 9: 517–527.

Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Endlicher, S. 1838. Genera plantarum. Asclepiadeae: 586–599. F. Beck, Vienna.

Endress, M. & P. Bruyns. 2000. A revised classification of the Apocynaceae s.l. Bot. Rev. (Lancaster) 66: 1–56.

———, B. Sennblad, S. Nilsson, L. Civeyrel, M. W. Chase, S. Huysmans, E. Grafstrom & B. Bremer. 1996. A phylogenetic analysis of the Apocynaceae s. str. and some related taxa in Gentianales, a multidisciplinary approach. Opera Bot. Belg. 7: 59–102.

Endress, P. K. 1990. Pattern of floral construction in ontogeny and phylogeny. Biol. J. Linn. Soc. 39: 153–175.

———. 1996. Diversity and Evolutionary Biology of Tropical Flowers. Cambridge Univ. Press, Cambridge.

———, M. Jenny & M. E. Fallen. 1983. Convergent elaboration of apocarpous gynoecia in higher advanced dicotyledons (Sapindales, Malvales, Gentianales). Nordic J. Bot. 3: 293–300.

Farris, J. S. 1969. A successive approximations approach to character weighting. Syst. Zool. 18: 374–385.

Forster, P. I. & K. Harold. 1989. *Secamone* R. Br. (Asclepiadaceae, Secamonoideae) in Australia. Austrobaileya 3: 69–78.

Friedman, F. 1990. Note sur la position systématique du genre *Trichosandra* Decne. (Asclepiadaceae). Adansonia 12: 131–138.

Goyder, D. J. 1992. *Secamone* (Asclepiadaceae subfam. Secamonoideae) in Africa. Kew Bull. 47: 437–474.

Hilu, K. W. & L. A. Alice. 1999. Evolutionary implications of *mat*K indels in Poaceae. Amer. J. Bot. 86: 1735–1741.

——— & H. Liang. 1997. The *mat*K gene: Sequence variation and application in plant systematics. Amer. J. Bot. 84: 830–839.

Johnson, L. A. & D. E. Soltis. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. Syst. Bot. 19: 143–156.

——— & ———. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *mat*K sequences. Ann. Missouri Bot. Gard. 82: 149–175.

———, J. L. Schultz & P. S. Soltis. 1996. Monophyly and generic relationships of Polemoniaceae based on *mat*K sequences. Amer. J. Bot. 83: 1207–1224.

Klackenberg, J. 1992a. Taxonomy of *Secamone s. lat.*

(Asclepiadaceae) in the Madagascar Region. Opera Bot. 112: 1–127.

———. 1992b. Taxonomy of *Secamone* (Asclepiadaceae) in Asia and Australia. Kew Bull. 47: 595–612.

———. 1995a. Taxonomy and phylogeny of the SE Asian genus *Genianthus* (Asclepiadaceae). Bot. Jahrb. Syst. 117: 401–467.

———. 1995b. Malgasy Asclepiadaceae, reinstatement of the genus *Pervillaea* and two new combinations. Phytologia 78: 189–191.

———. 1996a. The new genus *Calyptranthera* (Asclepiadaceae) from Madagascar. Novon 6: 25–28.

———. 1996b. Revision of the Malagasy genus *Pervillaea* (Asclepiadaceae) and its phylogenetic relationship to *Calyptranthera*. Nordic J. Bot. 16: 165–184.

———. 1997. Revision of the Malagasy genus *Calyptranthera* (Asclepiadaceae). Adansonia 19: 21–37.

———. 1998. *Calyptranthera gautieri* Klack (Apocynaceae s. l., Secamoneae), a new species from Madagascar. Candollea 53: 395–398.

———. In press. Notes on Secamonoideae (Apocynaceae) in Africa. Adansonia 23(2).

Kron, K. A., R. Fuller, D. M. Crayn, P. A. Gadek & C. J. Quinn. 1999. Phylogenetic relationships of epacrids and vaccinioids (Ericaceae s. l.) based on *mat*K sequence data. Pl. Syst. Evol. 218: 55–65.

Kunze, H. 1991. Structure and function in Asclepiad pollination. Pl. Syst. Evol. 176: 227–253.

Les, D. H., E. L. Schneider, D. J. Padgett, P. S. Soltis, D. E. Soltis & M. Zanis. 1999. Phylogeny, classification and floral evolution of water lilies (Nymphaeaceae; Nymphaeales): A synthesis of non-molecular, *rbc*L, *mat*K, and 18S rDNA data. Syst. Bot. 24: 28–46.

Li, J., A. L. Bogle & A. S. Klein. 1999. Phylogenetic relationships in the Hamamelidaceae: Evidence from the nucleotide sequences of the plastid gene *mat*K. Pl. Syst. Evol. 218: 205–219.

Liang, H. & K. W. Hilu. 1996. Application of the *mat*K gene sequences to grass systematics. Canad. J. Bot. 74: 125–134.

Liere, K. & G. Link. 1995. RNA binding activity of the *mat*K protein encoded by the chloroplast *trn*K intron from mustard (*Sinapis alba* L.). Nucl. Acid Res. 23: 917–921.

Manos, P. 1997. Phylogenetic analyses of higher Hamamelidaceae based on plastid sequence data. Amer. J. Bot. 84: 1407–1419.

Matsumoto, S., M. Kouchi, J. Kabuki, M. Kusunoki, Y. Ueda & H. Fukui. 1998. Phylogenetic analyses of the genus *Rosa* using the *mat*K sequence: Molecular evidence for the narrow genetic background of modern roses. Sci. Hort. 77: 73–82.

Mickevich, M. F. & S. J. Weller. 1990. Evolutionary characters analysis: Tracing character change on a cladogram. Cladistics 6: 137–170.

Neuhaus, H. & G. Link. 1987. The chloroplast $tRNA^{Lys}$ (UUU) gene from mustard (*Sinapis alba*) contains a class II intron potentially coding for a maturase-related polypeptide. Curr. Genet. 11: 251–257.

Nicholas, A. & H. Baijnath. 1994. A consensus classification for the order Gentianales with additional details on the suborder Apocynineae. Bot. Rev. 60: 410–482.

Nilsson, S., M. Endress & E. Grafstrom. 1993. On the relationship of the Apocynaceae and Periplocaceae. Grana Suppl. 2: 3–20.

Omlor, R. 1996. Do *Menabea venenata* and *Secamonopsis madagascariensis* represent missing links between Periplocaceae, Secamonoideae and Marsdenieae (Asclepiadaceae)? Kew Bull. 51: 695–715.

Plunkett, G. M., D. E. Soltis & P. S. Soltis. 1996. Evolutionary patterns in Apiaceae: Inferences based on *mat*K sequence data. Syst. Bot. 21: 477–496.

———, ——— & ———. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbc*L sequences data. Amer. J. Bot. 84: 565–580.

Potgieter, K. & V. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.

Puri, V. & R. Shiam. 1966. Studies in floral anatomy VIII. Vascular anatomy of the flower of the Asclepiadaceae with special reference to corona. Agra Univ. J. Res., Sci. 15: 189–216.

Rowe, N. P. & T. Speck. 1996. Biomechanical characteristics of the ontogeny and growth habit of the tropical liana *Condylocarpon guianense* (Apocynaceae). Int. J. Pl. Sci. 157: 406–417.

——— & ———. 1998. Biomechanics of plant growth forms: The trouble with fossil plants. Rev. Palaeobot. Palynol. 102: 43–62.

Safwat, F. W. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. Ann. Missouri Bot. Gard. 49: 95–129.

Sang, T., D. J. Crawford & T. F. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Amer. J. Bot. 84: 1120–1136.

Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.

——— & ———. 2000. Is there justification for differential a priori weighting in coding sequences? A case study from *rbc*L and Apocynaceae s.l. Syst. Biol. 49: 101–113.

———, M. E. Endress & B. Bremer. 1998. Morphology and molecular data in phylogenetic fraternity—The tribe Wrightieae (Apocynaceae) revisited. Amer. J. Bot. 85: 1143–1158.

Sibley, C. G. & J. E. Ahlquist. 1987. Avian phylogeny reconstructed from comparisons of the genetical material, DNA. Pp. 95–121 *in* C. Patterson (editor), Molecules and Morphology in Evolution: Conflict or Compromise? Cambridge Univ. Press, Cambridge.

Soltis, D. E., R. K. Kuzoff, E. Conti, R. Gornall & I. K. Ferguson. 1996. *mat*K and *rbc*L gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. Amer. J. Bot. 83: 371–382.

Speck, T. & N. P. Rowe. 1999. A quantitative approach to analytically defining size, growth form and habit in living and fossil plants. Pp. 447–479 *in* A. R. Hemsley & M. H. Kurmann (editors), The Evolution of Plant Architecture. Royal Botanic Gardens, Kew.

Steele, K. P. & R. Vilgalys. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. Syst. Bot. 19: 126–142.

Sugita, M., K. Shinozaki & M. Sugiura. 1985. Tobacco chloroplast $tRNA^{Lys}$ (UUU) gene contains a 2.56 kilobase-pair intron: An open reading frame and a conserved boundary sequence in the intron. Proc. Natl. Acad. Sci. U.S.A. 82: 3557–3561.

Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996. The subfamilial

and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.

Swofford, D. 1993. PAUP 3.1.1. Phylogenetic Analysis Using Parsimony. Smithsonian Institution, Washington, D.C.

Thiv, M., L. Struwe & J. W. Kadereit. 1999. The phylogenetic relationships and evolution of the Canarian laurel forest endemic *Ixanthus viscosus* (Aiton) Griseb. (Gentianaceae): Evidence from *mat*K and ITS sequences, and floral morphology and anatomy. Pl. Syst. Evol. 218: 299–317.

Verhoeven, R. L. & H. J. T. Venter. 1993. Pollen morphology of *Curroria*, *Mondia*, *Socotranthus* and *Stomatostemma* (Periplocaceae). Bothalia 23: 105–110.

——— & ———. 1994. Pollen morphology of the Periplocaceae from Madagascar. Grana 33: 295–308.

——— & ———. 1998. Pollinium structure in Periplocoideae (Apocynaceae). Grana 37: 1–14.

——— & ———. 2001. Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 569–582.

Wang, X.-R., Y. Tsumara, H. Yoshimaru, K. Nagasaka & A. E. Szmidt. 1999. Phylogenetic relationships of Eurasian pines (*Pinus*, Pinaceae) based on chloroplast *rbc*L, *mat*K, *rpl*20-*rps*18 spacer, and *trn*V intron sequences. Amer. J. Bot. 89: 1742–1753.

Wolfe, K. H. 1991. Protein-coding genes in chloroplast DNA: Compilation of nucleotide sequences, database entries and rates of molecular evolution. Pp. 467–482 *in* L. Bogorad & I. K. Vasil (editors), The Photosynthetic Apparatus: Molecular Biology and Operation 7B, Cell Culture and Somatic Cell Genetics in Plants. Academic Press, New York.

———, C. W. Morden & J. D. Palmer. 1991. Ins and outs of plastid genome evolution. Curr. Opin. Genet. Developm. 1: 523–529.

———, ——— & ———. 1992. Function and evolution of a minimal plastid genome from a non photosynthetic parasitic plant. Proc. Natl. Acad. Sci. U.S.A. 89: 10648–10652.

Woodson, R. E. & J. A. Moore. 1938. The vascular anatomy and comparative morphology of apocynaceous flowers. Bull. Torrey Bot. Club. 65: 135–166.

Xiang, Q.-Y., D. E. Soltis & P. S. Soltis. 1998. Phylogenetic relationships of Cornaceae and close relatives inferred from *mat*K and *rbc*L sequences. Amer. J. Bot. 85: 285–297.

Yokoyama, J., M. Suzuki, K. Iwatsuki & M. Hasebe. 2000. Molecular phylogeny of *Coriaria*, with special emphasis on the disjunct distribution. Molec. Phylogenet. Evol. 14: 11–19.

EVOLUTIONARY INNOVATION AND DIVERSIFICATION IN THE FLOWERS OF ASCLEPIADACEAE[1]

Mark Fishbein[2]

Abstract

Evolutionary innovation is an important mode of morphological diversification. Because explicit phylogenetic analyses are lacking for most evolutionary innovations, the patterns of origin, diversification, and homoplasy of innovations are poorly understood. Asclepiadaceae are a large angiosperm family characterized by a suite of putatively novel features that contributes to extreme floral complexity and diversity. In this paper, I use a preliminary phylogenetic hypothesis for Asclepiadaceae to explore the patterns of diversification in two novel floral characters, the pollinarium and the corona. The presence, number, and orientation of pollinia and the presence and form of corolline and gynostegial coronas are analyzed. Comparison of the histories of these structures suggests a contrast between relatively conserved evolution of pollinaria and labile evolution of coronas. I examine prior homology assessments of pollinaria and coronas and evaluate the sensitivity of evolutionary reconstructions to errors in homology assessment. These analyses point to crucial areas where additional ontogenetic studies, interpreted in a phylogenetic context, are required. This is particularly true in the phylogenetic assessment of the homology of corolline and gynostegial coronas. I also investigate the sensitivity of evolutionary reconstructions to phylogenetic uncertainty, and find this source of error to be slight.

Key words: Apocynaceae, Asclepiadaceae, character evolution, corona, diversification, innovation, novelty, phylogenetic uncertainty, pollinia.

Innovation is considered a central process in the evolutionary origin of morphological diversity (Mayr, 1960; Liem, 1974; Nitecki, 1990). Although the precise meaning of evolutionary innovation may vary from author to author, it generally refers to the appearance in a descendant of a new structure that differs "more than quantitatively" from its ancestral morphology (Mayr, 1960: 351). "Key" innovations have attracted special attention, because of their purported role in accelerating the rate of species diversification (Mitter et al., 1988; Farrell et al., 1991; Hodges, 1997). Despite keen interest in the role of evolutionary innovations in diversification, there has been remarkably little progress in understanding the ontogenetic bases of the origins of novelties and the evolutionary lability of novelties following their origin.

Species of Asclepiadaceae (including Periplocaceae) comprise a large clade of Apocynaceae sensu lato (Judd et al., 1999; Endress & Bruyns, 2000) that is notable for extreme floral complexity arising from several features that are rare or unknown outside of Apocynaceae s.l. Three floral structures merit particular attention due to their complexity and limited distribution among angiosperms: *pollinarium*, *gynostegium*, and *corona*. Each of these structures has been identified as a distinctive feature of Asclepiadaceae, although the presence of homologous structures (particularly gynostegia and coronas) in non-asclepiad Apocyna-

[1] Larry Hufford suggested the investigation of evolutionary novelties in asclepiad flowers. I thank Laure Civeyrel for generously sharing previously unpublished *mat*K sequences and for discussions about asclepiad phylogeny. Discussions with Mary Endress and Shelley McMahon about corolla-androecium synorganization and coronas were particularly illuminating. The comments of Mary Endress, Shelley McMahon, Ashley Nicholas, and Victoria Hollowell substantially improved the final version of the manuscript. I thank Jens Klackenberg for bringing his work on *Camptocarpus* to my attention. I am also grateful to Doug Soltis and Pam Soltis for supporting my molecular systematics research. Students in the Soltis lab, especially Mark Mort, generously assisted in optimizing techniques for obtaining *mat*K sequences. I am grateful to Chuck Cody, Washington State University, for maintaining research collections and to Chris Davitt and Val Lynch-Holm, WSU Electron Microscopy Center, for expert assistance. Permission to destructively sample herbarium specimens from WS is gratefully acknowledged. I thank Mary Endress and Doug Stevens for the invitation to participate in the symposium "Evolution and Phylogenetics of Apocynaceae s. l." at the XVI International Botanical Congress.

[2] School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236, U.S.A. Present address: Department of Biological Sciences, Mississippi State University, Mississippi State, Mississippi 39762-5759, U.S.A.

ceae has been discussed (Fallen, 1986; Kunze, 1990; Nilsson et al., 1993; Judd et al., 1994; Sennblad & Bremer, 1996; Endress & Bruyns, 2000).

The pollinarium (Bookman, 1981) of most Asclepiadaceae is composed of two or more *pollinia*, each of which contains all of the microspores of a single anther locule embedded in a hard matrix, and a *translator* apparatus, which develops from a stigmatic secretion and mechanically attaches the pollinia to a pollinator (Corry, 1883; Frye, 1902; Safwat, 1962; Schill & Jäkel, 1978; Kunze, 1993; Swarupanandan et al., 1996). Pollinarium structure has been the most important and consistently used character for the delimitation of major groups (subfamilies and tribes) of Asclepiadaceae (Brown, 1811; Endlicher, 1838; Decaisne, 1844; Schumann, 1895; Rosatti, 1989; Swarupanandan et al., 1996; Civeyrel et al., 1998). Studies of the development and mature structure of pollinaria have been carried out by Corry (1883), Frye (1902), Safwat (1962), Schill and Jäkel (1978), Kunze (1993), Civeyrel (1994), and Verhoeven and Venter (1997, 1998a, b, 2001 this volume). The evolution of the structure of pollinaria has been discussed by Safwat (1962), Wanntorp (1988), Kunze (1993, 1995a), Judd et al. (1994), Sennblad and Bremer (1996), Swarupanandan et al. (1996), and Civeyrel et al. (1998).

The gynostegium is composed of the postgenitally united stamens and carpels of the flower. The five stamens are typically connate throughout their length and joined by their anthers to the apex of the united styles. However, the filaments are free in Periplocoideae (Judd et al., 1994; Swarupanandan et al., 1996). Except for Periplocoideae, the encircling anthers completely block access to the stigmatic regions, except for narrow slits through which pollinia must enter to effect pollination (Kunze, 1991). Thus, pollinaria and gynostegia each exhibit a high degree of synorganization between androecium and gynoecium, with regard to both structure and function (Corry, 1883; Fallen, 1986; Endress, 1994). The evolution of the gynostegium has been well studied, particularly with respect to intermediate forms present in related Apocynaceae (Fallen, 1986; see also Wanntorp, 1988; Judd et al., 1994).

The corona is composed of one or more whorls of structures attached to, or located between, the corolla and androecium. Coronas are exceedingly diverse in Asclepiadaceae; they may be expressed as rings, scales, pads, or cups, often with several highly elaborated components present in the same flower (Kunze, 1990, 1995b, 1997; Liede & Kunze, 1993). Coronas have been the subject of structural and developmental studies (e.g., Frye, 1902; Hofmann & Specht, 1986; Kunze, 1990, 1995b, 1997; reviewed in Endress, 1994), and hypotheses of homology and transformation among corona types have been presented (Kunze, 1990; Liede & Kunze, 1993). Coronas have also been studied with respect to their functions in pollination (Wanntorp, 1974; Kunze, 1991, 1997; Endress, 1994). These functions include providing optical cues to pollinators (Endress, 1994), guiding insects into the proper position for pollinia removal and insertion by mechanically positioning proboscides (Kunze, 1991) or legs (Fishbein & Venable, 1996), and producing, conveying, and storing nectar (Galil & Zeroni, 1965; Kunze, 1990, 1997). However, the evolution of these structures has not been studied in an explicitly phylogenetic context.

Analysis of the evolution of corona morphology is hampered by uncertainty regarding the homology of corona structure and position across Asclepiadaceae. Although coronas have been the subject of intensive study, the conflicting interpretations of previous workers are surely the result of the exceptional diversity of forms and the seemingly fluid association of coronas with corolla and androecium in closely related taxa (e.g., Schumann, 1895; Woodson, 1941; Good, 1956; Hofmann & Specht, 1986; Kunze, 1990; Liede & Kunze, 1993; Nilsson et al., 1993). Liede and Kunze (1993; see also Kunze, 1990), echoing Woodson's (1941) and Good's (1956) attempts to bring consistency to the descriptions of coronas across Asclepiadaceae, concisely reviewed the uncertainties and discrepancies of past treatments and presented a typological framework for corona description. In addition, they presented a hypothetical transformation series linking their four basic corona types (Liede & Kunze, 1993, Fig. 1). In their descriptive system, a fundamental distinction was drawn between coronas of corolline derivation ("corolline" and "annular" types) and staminal derivation ("staminal" and "interstaminal" types). This distinction was previously emphasized by Good (1956). Kunze (1995b) emended this system by recognizing the dorsal anther appendages of some Gonolobinae as a fifth type of corona. The transformation series presented by Liede and Kunze (1993) implicitly assumed homology among their corona types, yet their discussion of particular coronas explicitly rejected the homology of different types. As recognized by Kunze (1990; Liede & Kunze, 1993), developmental studies would provide crucial data for homology assessment (Kaplan, 1984).

Phylogenetic and ontogenetic studies are required to more robustly assess the homology among the various structures termed "corona" in Asclepiadaceae (cf. Hufford, 1996a, b). In this paper, I analyze the evolution of the corolline, staminal, and interstaminal types, in order to focus on the origin and early diversification of coronas in Asclepiadaceae. For convenience, I group staminal and interstaminal coronas (Liede & Kunze, 1993) into "gynostegial" coronas, in order to emphasize and explore the homology between coronas that appear to be of staminal and corolline derivation (Schumann, 1895; Good, 1956; Kunze, 1990). Of the corona types of Liede and Kunze (1993; Kunze, 1990, 1995b), annular and dorsal coronas are of restricted distribution among apparently derived taxa (especially genera nested within Gonolobinae and Ceropegieae) and are not considered here.

I use a preliminary phylogenetic hypothesis for Asclepiadaceae to investigate the origin and diversification of pollinaria and coronas. The evolution of the gynostegium was achieved in large part in apocynaceous ancestors of Asclepiadaceae (Fallen, 1986) and will not be addressed here. For pollinaria and coronas I ask the following questions about the evolution of these structures: (1) Where in the phylogenetic history of Asclepiadaceae did pollinaria and coronas originate? (2) What are the patterns of diversification and homoplasy in pollinaria and coronas? and (3) What are the sensitivities of evolutionary reconstructions to uncertainties about homology and phylogenetic accuracy?

Materials and Methods

Structural data were gleaned from the literature and based to a lesser extent on my own observations. Character states were coded for exemplar taxa sampled for a preliminary phylogenetic analysis (Tables 1, 2; L. Civeyrel & M. Fishbein, unpublished data). Thorough study of character states present in larger clades was used to assess whether the coding of exemplars accurately reflected ancestral states of larger clades. The coding of coronal characters owes much to the careful observations of Kunze (1990, 1995b, 1997) and Endress (Nilsson et al., 1993; Endress & Bruyns, 2000), and to the conceptual framework of Liede and Kunze (1993; Kunze, 1990). Characters, their states, and a discussion of character coding are presented in Table 3. The evolution of pollinaria and coronas was analyzed by reconstructing the history of transformations under the criterion of maximum parsimony, as implemented in MacClade, version 3.05 (Maddison & Maddison, 1992).

The phylogenetic hypothesis used for the analysis of floral evolution was derived from published and unpublished analyses of chloroplast *mat*K sequences (Civeyrel et al., 1998; Civeyrel & Fishbein, unpublished data). The particular phylogeny on which the analyses were conducted resulted from a preliminary analysis of partial *mat*K sequences using maximum parsimony (Fig. 1). Analyses of complete sequences, voucher information, and methodological details will be published elsewhere. Species sampled and the higher taxa to which they belong (after Liede & Albers, 1994; Liede, 1997) are reported in Table 1. Outgroups were chosen from among the closest relatives of Asclepiadaceae in Apocynaceae s.l. (Civeyrel et al., 1998). Sensitivity of reconstructions of character evolution to a specific phylogenetic hypothesis was investigated by examining another published phylogeny based on chloroplast *rbc*L sequence data (Sennblad & Bremer, 1996). Consideration of this hypothesis is important because of the non-monophyly of Asclepiadaceae in the published analysis of *rbc*L sequences (Sennblad & Bremer, 1996; i.e., Periplocoideae are placed in a clade with non-asclepiad Apocynaceae, rather than as sister to Secamonoideae + Asclepiadoideae; see also Potgieter & Albert, 2001 this volume).

Results and Discussion

Origin and Diversification of Pollinia

The evolution of pollinia in Asclepiadaceae has been relatively conservative with regard to the presence and number of pollinia (Fig. 2). Little homoplasy was required for evolutionary reconstructions based on several schemes of weighting changes among character states (0, 2, or 4 pollinia per stamen). Assuming equal transition probabilities implies one or two origins of pollinia, outside of Periplocoideae. It is equally parsimonious to infer that pollinia originated once in the common ancestor of Asclepiadoideae and Secamonoideae or independently in these subfamilies. Under the former scenario, the ancestral pollinia number could be inferred with equal parsimony to be two or four per stamen, with either a subsequent change to four pollinia in Secamonoideae or two pollinia in Asclepiadoideae, respectively. Under the second scenario, pollinia of Asclepiadoideae and Secamonoideae would be convergent and not phylogenetically homologous (Patterson, 1982). However, the uniqueness of the asclepiad pollinium among angiosperms and the similarity between pollinia of Secamonoideae and Asclepiadoideae (Safwat, 1962; Schill & Jäkel, 1978; Civeyrel, 1994; Civeyrel et al., 1998;

Table 1. Taxa studied and their suprageneric classification (after Liede & Albers, 1994; Liede, 1997; cf. Endress & Bruyns, 2000).

Species	Subfamily (Tribe; Subtribe)
Ingroup	
Camptocarpus mauritianus (Lam.) Decne.	Periplocoideae
Cryptostegia grandiflora R. Br.	Periplocoideae
Hemidesmus indicus (L.) R. Br. ex Schult.	Periplocoideae
Periploca graeca L.	Periplocoideae
Raphionacme welwitschii Schltr. & Rendle	Periplocoideae
Schlechterella abyssinica (Chiov.) Venter & R. L. Verh.[a]	Periplocoideae
Pervillaea venenata (Baill.) Klack.	Secamonoideae
Secamone bosseri Klack.	Secamonoideae
Secamone parvifolia (Oliv.) Bullock	Secamonoideae
Secamone volubilis (Lam.) Marais	Secamonoideae
Secamonopsis madagascariensis Jum.	Secamonoideae
Fockea capensis Endl.	Asclepiadoideae (Fockeeae)
Dischidia formosana Maxim.	Asclepiadoideae (Marsdenieae)
Dregea sinensis Hemsl.	Asclepiadoideae (Marsdenieae)
Marsdenia edulis S. Watson	Asclepiadoideae (Marsdenieae)
Marsdenia laxiflora Donn. Sm.	Asclepiadoideae (Marsdenieae)
Stephanotis floribunda Brongn.	Asclepiadoideae (Marsdenieae)
Frerea indica Dalzell	Asclepiadoideae (Ceropegieae[b])
Huernia cf. *thuretii* Cels	Asclepiadoideae (Ceropegieae)
Riocreuxia burchellii K. Schum.	Asclepiadoideae (Ceropegieae)
Asclepias asperula (Decne.) Woodson	Asclepiadoideae (Asclepiadeae; Asclepiadinae)
Asclepias subaphylla Woodson	Asclepiadoideae (Asclepiadeae; Asclepiadinae)
Asclepias virletii E. Fourn.	Asclepiadoideae (Asclepiadeae; Asclepiadinae)
Asclepias zanthodacryon (L. B. Sm.) Woodson	Asclepiadoideae (Asclepiadeae; Asclepiadinae)
Pergularia daemia (Forssk.) Chiov.	Asclepiadoideae (Asclepiadeae; Asclepiadinae)
Tylophora indica (Burm. f.) Merr.	Asclepiadoideae (Asclepiadeae; Astephaninae[c])
Vincetoxicum nigrum (L.) Moench	Asclepiadoideae (Asclepiadeae; Astephaninae[c])
Gonolobus arizonicus (A. Gray) Woodson	Asclepiadoideae (Asclepiadeae; Gonolobinae)
Gonolobus sp. nov. aff. *uniflorus* HBK	Asclepiadoideae (Asclepiadeae; Gonolobinae)
Gonolobus xanthotrichus Brandegee	Asclepiadoideae (Asclepiadeae; Gonolobinae)
Matelea sp. nov. aff. *parvifolia* (Torr.) Woodson	Asclepiadoideae (Asclepiadeae; Gonolobinae)
Matelea quirosii (Standl.) Woodson	Asclepiadoideae (Asclepiadeae; Gonolobinae)
Schubertia grandiflora Mart. & Zucc.	Asclepiadoideae (Asclepiadeae; Gonolobinae)
Cynanchum laeve (Michx.) Pers.	Asclepiadoideae (Asclepiadeae; Metastelminae)
Cynanchum palmeri (S. Watson) S. F. Blake	Asclepiadoideae (Asclepiadeae; Metastelminae)
Cynanchum utahense (Englem.) Woodson	Asclepiadoideae (Asclepiadeae; Metastelminae)
Pentarrhinum insipidum E. Mey.	Asclepiadoideae (Asclepiadeae; Metastelminae)
Sarcostemma clausum (Jacq.) Schult.	Asclepiadoideae (Asclepiadeae; Metastelminae)
Araujia sericifera Brot.	Asclepiadoideae (Asclepiadeae; Oxypetalinae)
Morrenia odorata (Hook. & Arn.) Lindl.	Asclepiadoideae (Asclepiadeae; Oxypetalinae)
Outgroup	
Apocynum androsaemifolium L.	
Strophanthus divaricatus (Lour.) Hook. & Arn.	

[a] Reported as *Triodoglossum abyssinicum* (Chiov.) Bullock by Civeyrel et al. (1998); included in *Raphionacme* by Venter and Verhoeven (1997); see Venter and Verhoeven (1998).

[b] Ceropegieae Orb. has priority over Stapelieae Decne. and should be adopted as the tribal name (J. Reveal, *Index Nominum Suprageneroricum Plantarum Vascularium*, <http://matrix.nal.usda.gov:8080/star/suprageneriename.html>, consulted 11 February 2000).

[c] Removed from Astephaninae by Liede (2001 this volume).

Table 2. Matrix of character states of pollinia and coronas for the taxa in Table 1. Character names, state names, and discussion of character coding are presented in Table 3.

Taxon	1	2	3a	3b	4a	4b
Camptocarpus	0	0	1	0	0	1
Cryptostegia	0	0	1	1	0	0
Hemidesmus	1	0	1	1	0	0
Periploca	0	0	1	1	0	0
Raphionacme	0	0	1	0	0	1
Schlechterella	1	0	1	0	0	1
Pervillaea	1	2	0	0	1	1
Secamone bosseri	1	2	0	0	1	1
Secamone parvifolia	1	2	1	1	1	1
Secamone volubilis	1	2	0	0	1	1
Secamonopsis	1	2	1	1	1	1
Fockea	2	2	0	0	2	2
Dischidia	2	2	0	0	1	1
Dregea	2	?	0	0	1	1
Marsdenia edulis	2	2	0	0	1	1
Marsdenia laxiflora	2	2	0	0	1	1
Stephanotis	2	2	0	0	1	1
Frerea	2	2	0	0	2	2
Huernia	2	2	0	0	2	2
Riocreuxia	2	2	0	0	2	2
Asclepias asperula	2	1	0	0	2	2
Asclepias subaphylla	2	1	0	0	2	2
Asclepias virletii	2	1	0	0	2	2
Asclepias zanthodacryon	2	1	0	0	2	2
Pergularia	2	1	0	0	2	2
Tylophora	2	1	0	0	2	2
Vincetoxicum	2	1	0	0	2	2
Gonolobus arizonicus	2	1	0	0	2	2
Gonolobus sp. nov. aff. *uniflorus*	2	1	0	0	2	2
Gonolobus xanthotrichus	2	1	0	0	2	2
Matelea sp. nov. aff. *parvifolia*	2	1	0	0	2	2
Matelea quirosii	2	1	0	0	2	2
Schubertia	2	1	0	0	2	2
Cynanchum laeve	2	1	0	0	1	1
Cynanchum palmeri	2	1	0	0	2	2
Cynanchum utahense	2	1	0	0	0	0
Pentarrhinum	2	1	0	0	2	2
Sarcostemma	2	1	0	0	2	2
Araujia	2	1	0	0	2	2
Morrenia	2	1	0	0	2	2
Apocynum	0	0	1	1	0	0
Strophanthus	0	0	1	1	0	0

Verhoeven & Venter, 2001) suggest that the hypothesis of a single origin of pollinia is to be favored. Any step matrix in which the origin of pollinia is less likely (no matter how slightly) than a change between two and four pollinia per stamen favors a single origin of pollinia.

Consideration of the structure of anthers in Periplocoideae and non-asclepiad Apocynaceae favors the reconstruction of four, rather than two, pollinia at the time of origin. The number of pollinia per stamen is a direct consequence of the number of fertile anther locules (Safwat, 1962; Swarupanandan et al., 1996). As in most angiosperms, all Apocynaceae, except Asclepiadoideae, have four-locular anthers. The inference of an ancestral number of two pollinia per stamen would require a less parsimonious reversal from two anther locules to four in Secamonoideae, rather than retention of the ancestral condition. Thus, a single origin of pollinia in the common ancestor of Asclepiadoideae and Secamonoideae, in which four pollinia were produced by four-locular anthers, is strongly favored over alternate hypotheses. According to this scenario, presence of pollinia is a synapomorphy of Asclepiadoideae + Secamonoideae and reduction from four to two pollinia per stamen is a synapomorphy of Asclepiadoideae (Judd et al., 1994). It should also be noted that pollinia of some Secamonoideae may be united, giving the appearance of one or two pollinia per pollinarium, but this condition is clearly derived and secamonoid anthers are invariably four-locular (Civeyrel, 1994; Civeyrel et al., 1998; Endress & Bruyns, 2000).

Pollinia have been reported from as many as seven genera of Periplocoideae (Schill & Jäkel, 1978; Nilsson et al., 1993; Verhoeven & Venter, 1994, 1998a, b, 2001; Venter & Verhoeven, 1997; Endress & Bruyns, 2000), although these have been reduced to six genera in the most recent classification (Venter & Verhoeven, 2001 this volume). The rigorous comparative study of the pollen morphology of asclepiads by Verhoeven and Venter (1998b, 2001) summarizes the salient similarities and differences among pollinia of Periplocoideae, Secamonoideae, and Asclepiadoideae. Pollinia of Periplocoideae and Secamonoideae retain two ancestral features that are modified in most Asclepiadoideae (with the notable exception of *Fockea* Endl.): production of pollen tetrads and the absence of a pollinium wall. However, pollinia of Periplocoideae lack a derived feature common to all examined Secamonoideae and Asclepiadoideae—the reduction of the inner wall layers of pollen tetrads. Among Periplocoideae bearing pollinia, all genera are Asian, except African *Schlechterella* K. Schum. (syn. *Triodoglossum* Bullock), which differs in producing multiporate tetrads (Verhoeven & Venter, 1998a). This difference led Verhoeven and Venter (2001) to predict that the origins of pollinia in Asia and Africa were independent. From the foregoing observations, one may draw the following conclusions regarding the phylogenetic homology of pollinia of asclepiads: (1) pollinia of Asclepiadoideae and Secamono-

Table 3. Character names, state names, and discussion of character coding. All characters are treated as unordered in evolutionary reconstructions using maximum parsimony. The effect of relaxation of this assumption for character 1 is discussed in the text.

1. Number of pollinia per stamen: none (0), four (1), two (2). Pollen morphology of Asclepiadaceae has been surveyed and summarized by Brown (1811), Corry (1883), Schill and Jäkel (1978), Swarupanandan et al. (1996), Civeyrel (1994; Civeyrel et al., 1998), and Verhoeven and Venter (1998b, 2001). Coding of the number of pollinia is unambiguous for Asclepiadoideae and Secamonoideae. Pollen grains of Periplocoideae are typically shed in tetrads. However, pollinia have been reported (Schill & Jäkel, 1978; Verhoeven & Venter, 1994, 1998a, 1998b, 2001; Venter & Verhoeven, 1997). These pollinia differ from those of Asclepiadoideae and Secamonoideae in their softer consistency and well-developed inner walls of the tetrads (Endress & Bruyns, 2000; Verhoeven & Venter, 2001). However, pollinia of Periplocoideae are similar to those of the Secamonoideae and *Fockea* (Asclepiadoideae) in consisting of tetrads and lacking a distinct pollinium wall (Verhoeven & Venter, 2001). With the limited sampling of Periplocoideae in the present study, pollinia are reconstructed as independently derived in Periplocoideae (in *Hemidesmus* and *Schlechterella*) and remaining Asclepiadaceae. Two origins of pollinia were hypothesized for Periplocoideae by Verhoeven and Venter (2001), with one origin putatively homologous to the pollinia of Secamonoideae and Asclepiadoideae. However, the sparse distribution of genera containing pollinia across the recognized tribes of Periplocoideae (Venter & Verhoeven, 1997) suggests that multiple origins (and perhaps even losses) of pollinia might be inferred. A more refined understanding of the phylogeny of Periplocoideae will undoubtedly yield important insights into the homology and evolution of their pollinia.

2. Position of attachment of translator to pollinia: pollinia absent (0), attachment apical (1), attachment basal (2). Translator attachment of Periplocoideae is coded as absent, because the mechanism in this subfamily is via adhesion, following flower maturation. This condition differs substantially from Secamonoideae and Asclepiadoideae, in which attachment of pollinia to the translator occurs much earlier in development, by a different mechanism (Brown, 1811; Safwat, 1962; Kunze 1993; Swarupanandan et al., 1996). The classic division of Asclepiadoideae into three tribes was based on the orientation of the pollinium relative to the translator: pendent, horizontal, or erect (Brown, 1811; Endlicher, 1838). Subsequently, this division was challenged as artificial due to imprecision in the description of the point of attachment of translators to pollinia (Woodson, 1941; Swarupanandan et al., 1996). I have coded the orientation of the pollinium relative to the translator following Swarupanandan et al. (1996), although their homology criterion has recently come under question (Endress & Bruyns, 2000).

3a. Corolline corona: absent (0), present (1). The definition of this character follows the description of Liede and Kunze (1993; see also Kunze, 1990; Nilsson et al., 1993). The primary homology criterion is topological. Corolline coronas are composed of five distinct elements positioned on the corolla, proximal to and radially aligned with the corona sinuses. This definition explicitly excludes annular coronas, which are composed of united segments, or are unsegmented (Woodson, 1941; Good, 1956; Liede & Kunze, 1993). Character state coding differs from that of character 3b only for Periplocoideae. Several genera of this subfamily have contradictory or ambiguous descriptions of the position of corona elements (i.e., corolline vs. gynostegial; Kunze, 1990; Nilsson et al., 1993; Klackenberg, 1998). In coding character 3a, I have assumed that corolline coronas are present in all Periplocoideae that have coronas, following the observations of Kunze (1990) and Klackenberg (1998). Other taxa were coded based upon the observations of Hooker (1883), Woodson (1941), Sundell (1981), Rosatti (1989), Kunze (1990, 1997), Klackenberg (1992a, 1992b, 1995), Li et al. (1995), Civeyrel and Klackenberg (1996), Forster (1996), Liede (1996), and personal observations.

3b. Corolline corona: absent (0), present (1). Coding of this character is identical to that in character 3a, except that *Raphionacme*, *Schlechterella*, and *Camptocarpus* are coded as lacking corolline coronas. Coronas in these genera are assumed to be positioned on the gynostegium, as observed by Nilsson et al. (1993) in *Raphionacme*.

4a. Gynostegial corona: absent (0), composed of free staminal segments (1), composed of united staminal and interstaminal segments (2). The definition of this character follows Liede and Kunze (1993; see also Kunze, 1990). Dorsal anther coronas of Gonolobinae (Kunze, 1995b) are specifically excluded. Gynostegial coronas include those coronas that are attached to the staminal column, but not the corolla. In cases where the corona is attached to both the androecium and corolla, a gynostegial corona is coded as present if the attachment to the column is clearly much greater than to the corolla. Ambiguous cases in Periplocoideae are treated with separate codings, as in characters 3a and 3b. In character 4a, all Periplocoideae are coded as lacking gynostegial coronas (cf. character 3a; Kunze, 1990; Klackenberg, 1998). Gynostegial coronas have been subdivided into those composed of five distinct segments aligned radially with the staminal filaments and lacking any discernible union among segments ("staminal") and those composed of segments that are united at least basally, sometimes forming a cylinder around the gynostegium, and often with apically free segments that are in positions both aligned with the filaments and alternating with them ("united staminal and interstaminal"). These latter coronas may achieve a high degree of complexity, appearing to be constructed of multiple whorls of segments (e.g., *Frerea*, Ceropegieae). Observations are based on the sources listed in the discussion of character 3a.

4b. Gynostegial corona: absent (0), composed of free staminal segments (1), composed of united staminal and interstaminal segments (2). Coding of this character is identical to that in character 4a, except that *Raphionacme*, *Schlechterella*, and *Camptocarpus* are coded as possessing gynostegial coronas composed of free staminal segments, as observed by Nilsson et al. (1993) in *Raphionacme*.

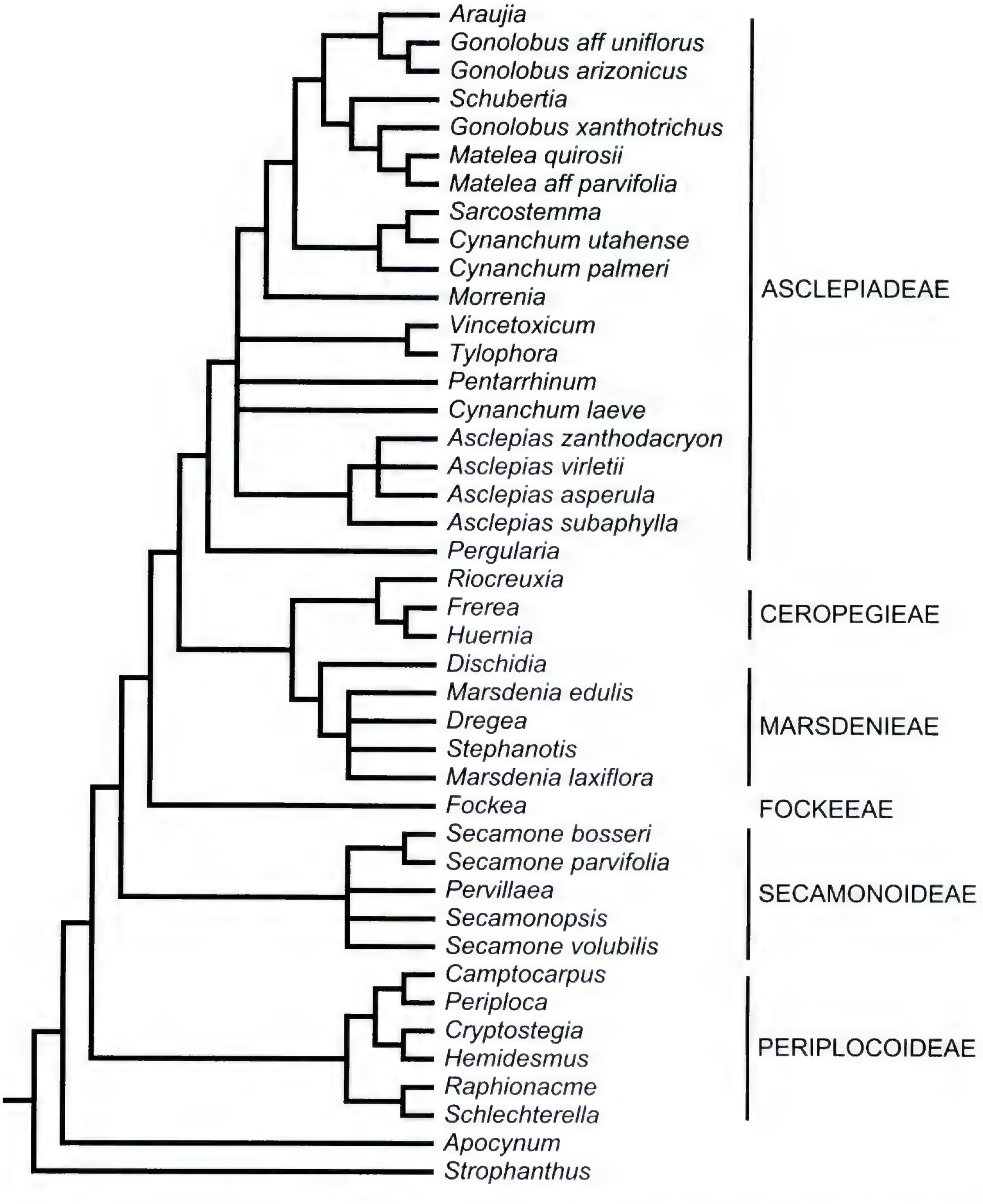


Figure 1. Phylogenetic hypothesis of broad-scale relationships in Asclepiadaceae based on maximum parsimony analysis of partial and complete *mat*K sequences (L. Civeyrel & M. Fishbein, unpublished; cf. Civeyrel et al., 1998). Subfamilial and tribal membership is indicated for groups supported as clades. Asclepiadoideae, consisting of Asclepiadeae, Ceropegieae, Marsdenieae, and Fockeeae, are monophyletic, but are not labeled.

ideae are likely homologous; and (2) pollinia of some or all Periplocoideae *may* be homologous to those of other asclepiads; however, the imperfect understanding of the phylogeny of Periplocoideae at present prevents assessment of this hypothesis. With the limited sampling in the present study, pollinia of Periplocoideae are not reconstructed as homologous with those of other asclepiads. If current taxonomy (Venter & Verhoeven, 1997) reflects phylogenetic history, it appears that pollinia may have evolved independently on several occasions in Periplocoideae, based on the distribution of

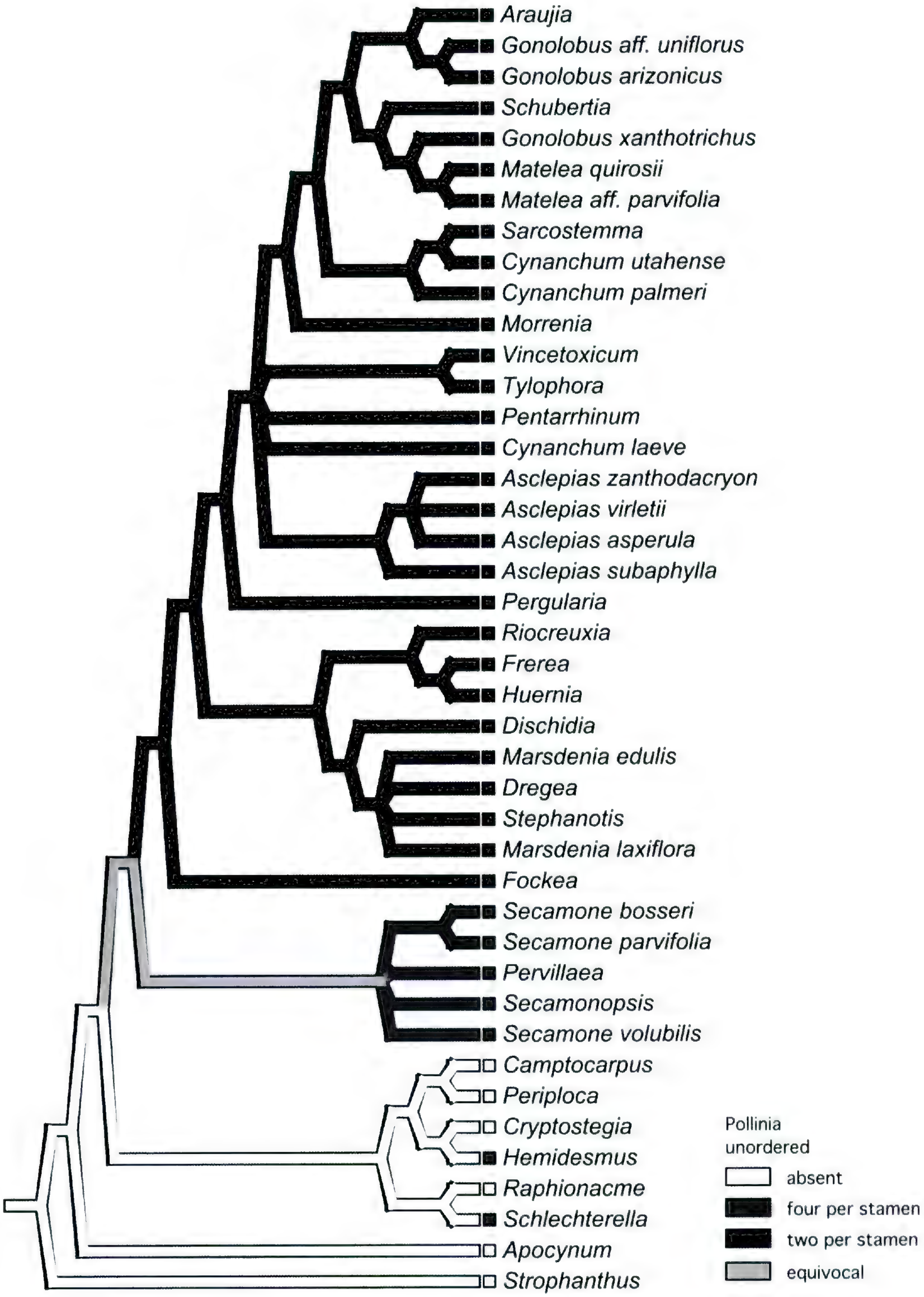


Figure 2. Maximum parsimony reconstruction of the evolutionary history of pollinia number, based on the cladogram in Figure 1. Character states are treated as unordered with equal transition probabilities among states. Ancestors and branches reconstructed as equivocal are inferred with equal parsimony to be lacking pollinia or to possess two or four pollinia per stamen. Additional reconstructions found with alternate transition probabilities are discussed in the text.

pollinia among putatively isolated genera in each of the three recognized tribes. If pollinia among most Periplocoideae are homologous, then multiple reversions to free tetrads must be inferred. The contrast between the lability of pollen aggregation in Periplocoideae and fixity in remaining asclepiads is noteworthy. Future studies evaluating the phylogenetic homology of pollinia, the number of gains (and losses?), and possible selective advantages would be of great interest.

Pollinium orientation has been used as a diagnostic character for suprageneric taxa recognized within Asclepiadoideae (e.g., Endlicher, 1838; reviewed in Rosatti, 1989; Swarupanandan, 1996; Civeyrel et al., 1998). Often, three tribes have been recognized defined by erect (Marsdenieae; note that this group has received a variety of tribal names), horizontal (Gonolobeae), and pendulous (Asclepiadeae) pollinia. Ceropegieae (Stapelieae of most modern authors, e.g., Liede & Albers, 1994), which are often recognized as an additional tribe, share erect pollinia with Marsdenieae. However, the diagnostic value of pollinia orientation was questioned due to the presence of intermediate or seemingly discordant orientations of some species, especially in Gonolobineae (Woodson, 1941; Rosatti, 1989; Kunze, 1995b; Liede, 1996; Swarupanandan et al., 1996; Civeyrel et al., 1998; Endress & Bruyns, 2000). Swarupanandan et al. (1996) reevaluated this partitioning of pollinium orientation and presented refined categories, emphasizing the importance of the orientation of the anther locule and the position of attachment of the translator. Thus, the horizontally oriented pollinia of Gonolobeae have morphologically equivalent translator attachments as the pendulous pollinia of Asclepiadeae. Swarupanandan et al. (1996) advocated using multiple criteria to evaluate whether the translator is attached to the part of the pollinium positioned apically or basally in the locule. I have adopted these criteria for analyzing the evolution of pollinium orientation, bearing in mind the exceptions noted by Endress and Bruyns (2000). Also, I have treated the very different adhesive attachment of pollinia to translators in Periplocoideae as nonhomologous (see Table 3).

As with pollinia number per stamen, the evolution of the position of translator attachment is reconstructed to have been conservative (Fig. 3). Assuming that pollinia originated once in the common ancestor of Secamonoideae and Asclepiadoideae (see above), the ancestral point of attachment is unambiguously reconstructed to be basal. This orientation is retained in Secamonoideae and several lineages of Asclepiadoideae: Fockeeae, Marsdenieae, and Ceropegieae. A single change from basal to apical translator attachment is inferred in the ancestor of Asclepiadeae; no reversals are apparent among the representatives of this large tribe. Thus, no homoplasy is evident in the single most parsimonious reconstruction. It should be noted, however, that the sparse sampling in the present study may obscure homoplasious convergence to apical attachment among clades coded as uniformly basal or (more likely) reversal to basal attachment in Asclepiadeae (Endress & Bruyns, 2000). A rigorous basis for assessing the homology of ambiguous cases would be a comparative ontogenetic study of pollinaria (cf. Safwat, 1962; Kunze, 1993).

ORIGIN AND DIVERSIFICATION OF CORONAS

Corolline coronas. Corolline coronas are defined by structural and topological criteria: (1) they are composed of five distinct segments; and (2) they are positioned on the corolla, aligned with the sinuses of the limb (Good, 1956; Kunze, 1990; Nilsson et al., 1993). My analysis of corolline coronas explicitly excludes annular coronas (Liede & Kunze, 1993), which are conspicuous in a number of Gonolobinae and Ceropegieae, but do not appear to be homologous (see Endress & Bruyns, 2000, for a contrasting interpretation). Corolline coronas have been considered to be prevalent in Periplocoideae, but very uncommon in Asclepiadoideae (Kunze, 1990; Liede & Kunze, 1993; Nilsson et al., 1993). They have been reported from Secamonoideae, but their distribution in this subfamily has been little discussed (Safwat, 1962; Kunze, 1990). Corolline coronas are also found widely among non-asclepiad Apocynaceae (Kunze, 1990; Endress & Bruyns, 2000). The taxonomic distribution of corolline coronas has suggested that they are ancestral in Asclepiadaceae (Good, 1956; Kunze, 1990; Liede & Kunze, 1993). Although most Periplocoideae are described as having corolline coronas, some genera possess coronas that, while similar in form, appear to be more closely associated with the stamens than the corolla, e.g., *Raphionacme* Harv. (including *Schlechterella*) and *Finlaysonia* Wall. (Kunze, 1990; Nilsson et al., 1993; Forster, 1996; Venter & Verhoeven, 1997; see also Endress & Bruyns, 2000). In *Raphionacme*, ambiguity is due to the attachment of the corona segments near the point of divergence of stamens and corolla (Kunze, 1990; Nilsson et al., 1993). To account for this ambiguity, two codings of the presence of corolline coronas among Periplocoideae were explored (Tables 2, 3). In character 3a, I coded the coronas of all exemplars of Periplocoideae as being corolline, as im-

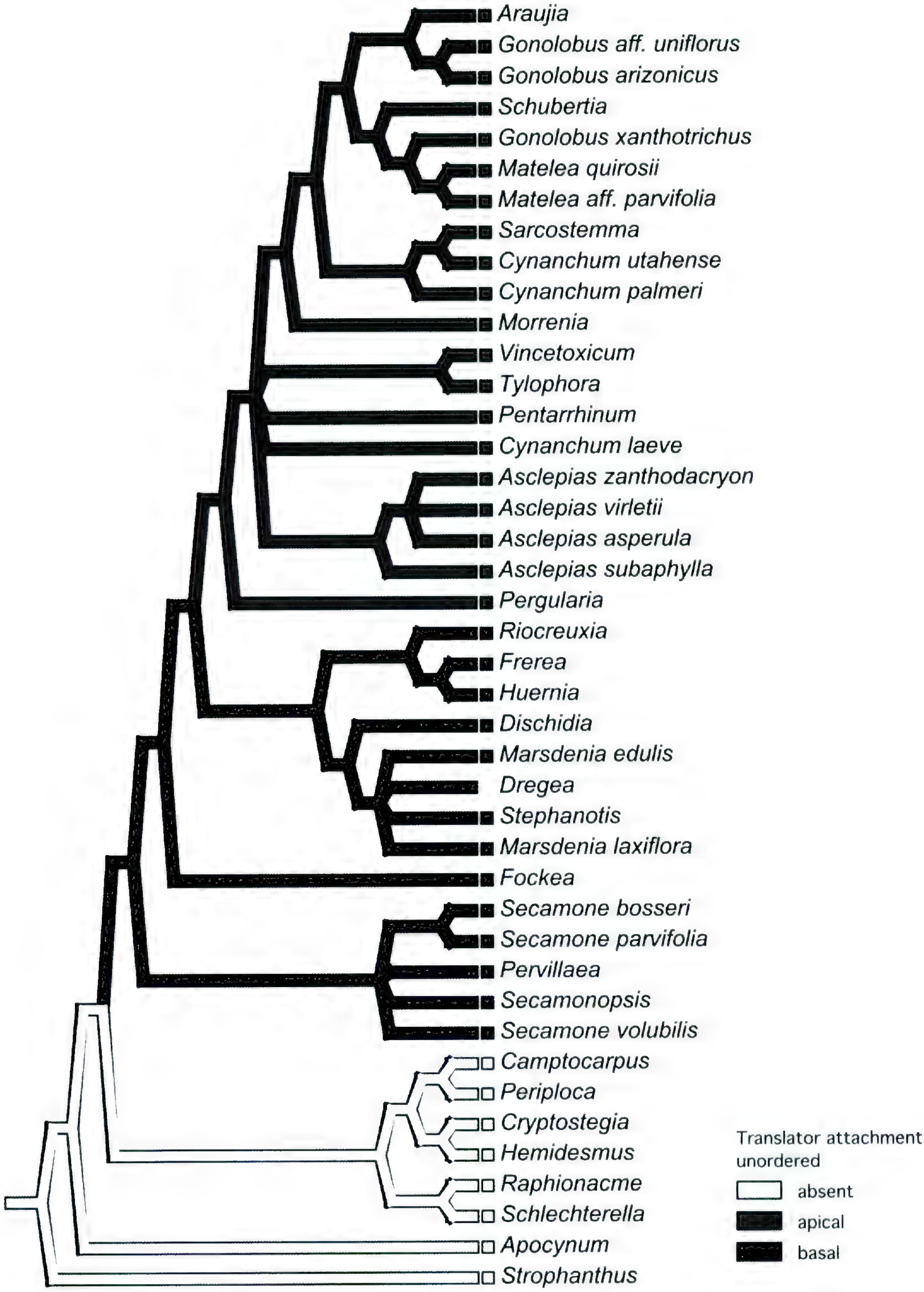


Figure 3. Maximum parsimony reconstruction of the evolutionary history of position of translator attachment to the pollinium, based on the cladogram in Figure 1. Character states are treated as unordered with equal transition probabilities among states.

plied by Kunze (1990). In character 3b, I coded all exemplars of Periplocoideae with ambiguous corona position (*Raphionacme, Schlechterella, Camptocarpus* Decne.) as lacking corolline coronas (but possessing gynostegial coronas; see below).

Parsimony reconstruction of the evolutionary history of corolline coronas, assuming their uniform presence in Periplocoideae (character 3a), requires little homoplasy (Fig. 4). The common ancestor of Asclepiadaceae is inferred to possess a corolline corona, which is a retained plesiomorphy present in related Apocynaceae (cf. Good, 1956; Kunze, 1990; Liede & Kunze, 1993). This ancestral corolline corona is retained in Periplocoideae, and no losses of corolline coronas are inferred among the sampled taxa. Loss of the corolline corona may have occurred at least once in Periplocoideae, as they have been reported to be lacking in three unsampled genera (*Baseonema* Schltr. & Rendle, *Baroniella* Costantin & Gallaud, *Phyllanthera* Blume; Venter & Verhoeven, 1997). However, Klackenberg (1997) has interpreted the corona in these genera to be a unique corolline form in which the segments occur in a novel position aligned with the corolla lobes. If interpreted correctly, such coronas would represent a distinct form not easily homologized with corolline or other gynostegial forms.

Asclepiadoideae uniformly lack corolline coronas (but see reports of secamonoid-like ridges in several genera of Marsdenieae in Hooker, 1883; Good, 1956; Kunze, 1990; Goyder, 1994; Li et al., 1995; Forster, 1996). Whether this absence is synapomorphic for Asclepiadoideae is equivocal, due to equally parsimonious reconstructions of the common ancestor of Asclepiadoideae and Secamonoideae. The ambiguity arises because of variation in the presence of corolline coronas among Secamonoideae (Safwat, 1962; Kunze, 1990; Klackenberg, 1992a, b, 1995; Li et al., 1995; Civeyrel & Klackenberg, 1996; Forster, 1996). Two of the five exemplars of this subfamily are coded as possessing corolline coronas. The phylogenetic placement of these exemplars results in an equivocal reconstruction of corolline corona presence in the common ancestor of Secamonoideae, which results in the ambiguity deeper in the phylogeny. Thus, sampling of Secamonoideae plays a crucial role in the inference of whether Secamonoideae or Asclepiadaceae + Secamonoideae ancestrally possessed a corolline corona. It is not possible at this time to predict whether more complete sampling would favor the presence or absence of corolline coronas in these ancestors. Thus, the exact point in the phylogeny where the loss of the corolline corona in Asclepiadaceae occurred cannot be determined (i.e., whether it occurred in the immediate ancestor of Asclepiadoideae or the common ancestor of Asclepiadoideae and Secamonoideae). Further, it cannot be determined whether any or all corolline coronas present in Secamonoideae are retained from the plesiomorphic presence in Periplocoideae and non-asclepiad Apocynaceae, or represent one or more instances of regaining lost coronas. A hint that corolline coronas are not homologous in Secamonoideae and Periplocoideae is that corolline coronas of Secamonoideae (and similar coronas of Asclepiadoideae) are invariably described as "pads," "ridges," and "cushions" (Kunze, 1990; Li et al., 1995; Forster, 1996), rather than as scales and filaments typical of Periplocoideae (Kunze, 1990; Nilsson et al., 1993; Klackenberg, 1998). Although positioned similarly, structural differences place the homology of these coronas in doubt.

Allowing for the absence of corolline coronas in some Periplocoideae (character 3b, Tables 2, 3) results in greater ambiguity in ancestral state reconstructions and a higher level of homoplasy (Fig. 5). The presence of a corolline corona in the ancestors of Asclepiadaceae, Periplocoideae, Secamonoideae, and Asclepiadoideae + Secamonoideae is equivocal in each case. This greater degree of uncertainty is due entirely to variation in the presence of corolline coronas among Periplocoideae. As a result, it is equally parsimonious to infer that those Periplocoideae with corolline coronas have retained them from non-asclepiad relatives, or that they have evolved convergently. Again, sampling may affect whether the presence of a corolline corona is most parsimoniously inferred to be plesiomorphic or convergent. Because most genera of Periplocoideae have coronas that are clearly of the corolline type (Kunze, 1990; Nilsson et al., 1993; Venter & Verhoeven, 1997), it is possible that additional sampling would favor reconstructions in which Periplocoideae retain an ancestral corolline corona. Such a scenario implies at least two independent losses of corolline coronas within Periplocoideae (Fig. 5), in addition to the putative cases discussed above.

Gynostegial coronas. Gynostegial coronas exhibit a far greater range of diversity of form than corolline coronas. My use of "gynostegial" corona follows Liede and Kunze (1993) and is largely equivalent to the "true" corona of Woodson (1941) and the "staminal" corona of Schumann (1895), Good (1956), and Kunze (1990). I coded gynostegial coronas as exhibiting two basic forms: separate staminal segments (Fig. 6) and united staminal and interstaminal segments (Fig. 7). Although Liede and Kunze (1993) distinguished staminal and interstaminal corona segments as fundamentally dis-

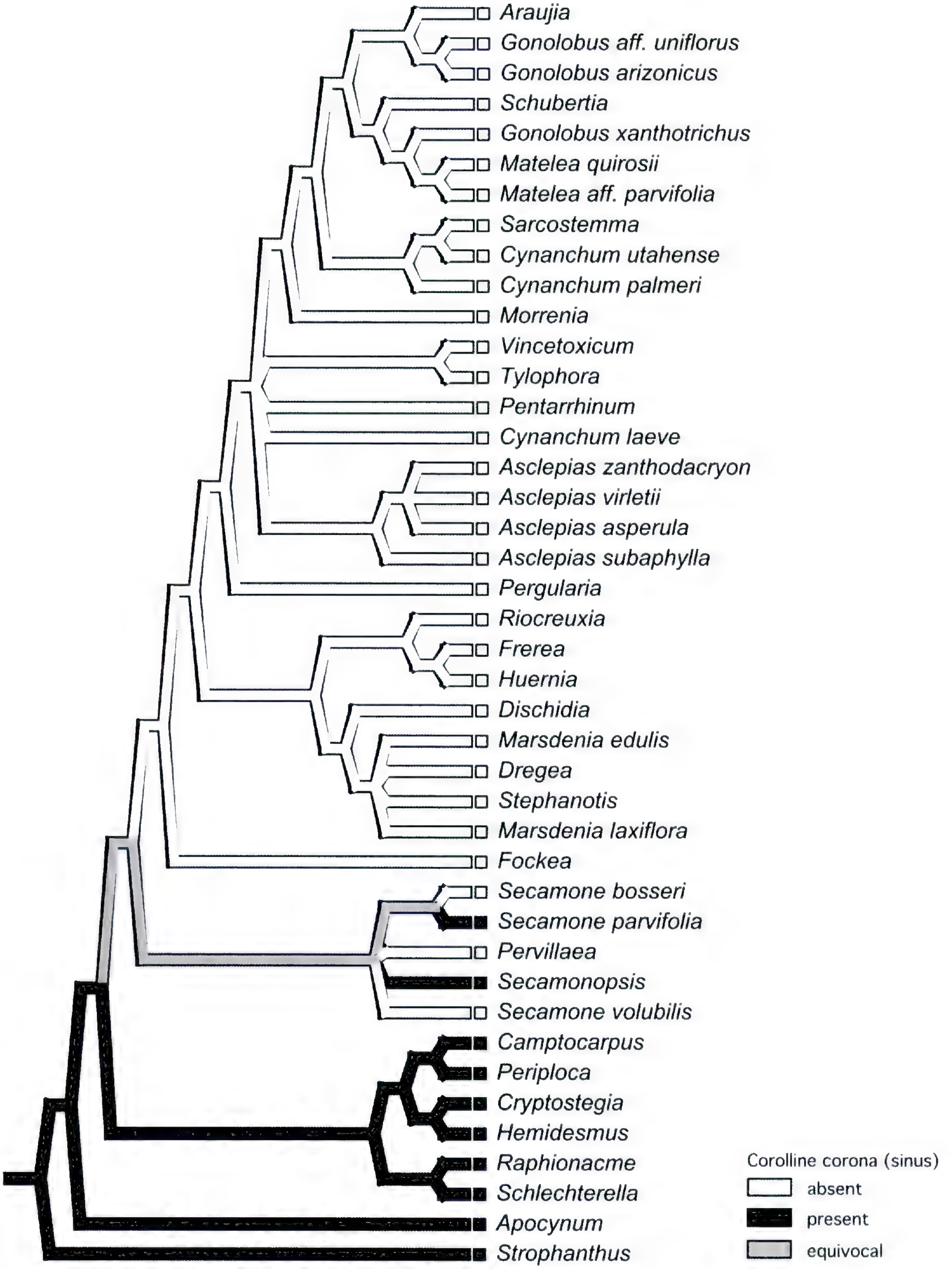


Figure 4. Maximum parsimony reconstruction of the evolutionary history of corolline coronas, based on the cladogram in Figure 1 and the coding of character 3a (Tables 2, 3).

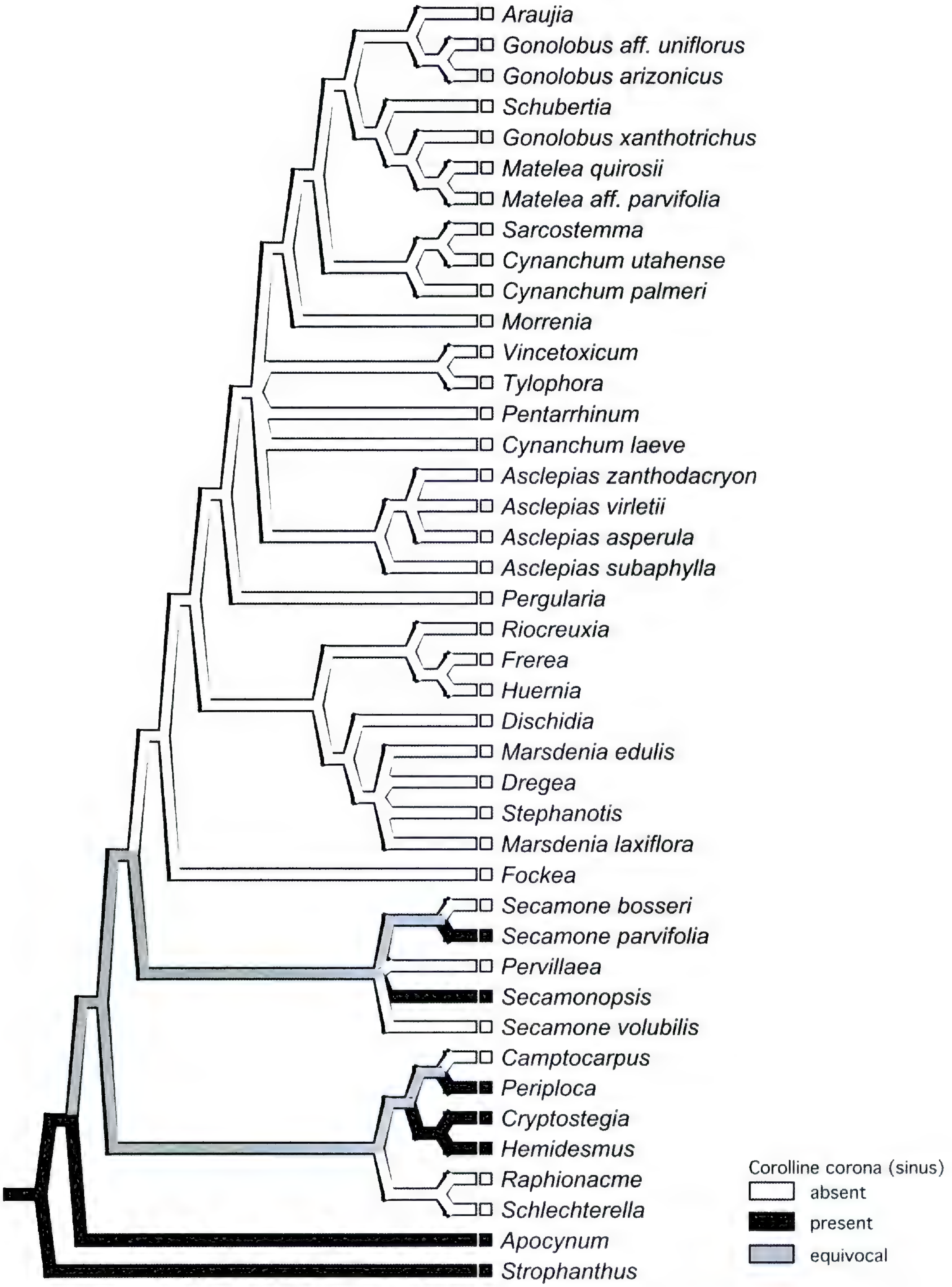
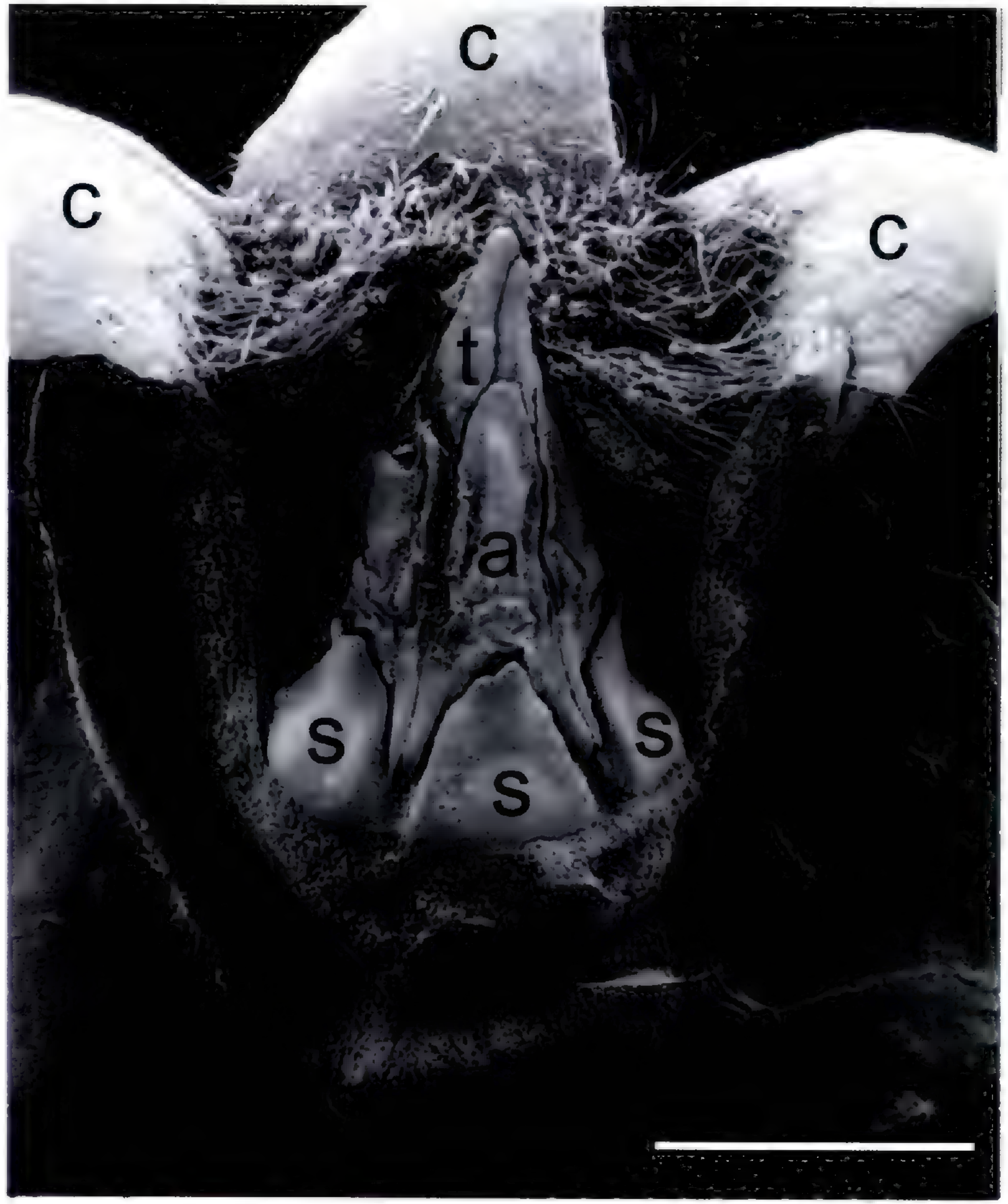


Figure 5. Maximum parsimony reconstruction of the evolutionary history of corolline coronas, based on the cladogram in Figure 1 and the coding of character 3b (Tables 2, 3).



Figures 6, 7. Scanning electron micrographs of mature flowers showing gynostegial coronas. Flowers are viewed obliquely from the side. Portions of the perianth (Figs. 6, 7) and corona segments (Fig. 7 only) were removed to reveal hidden features. Abbreviations: a = connate anthers, c = corolla, i = interstaminal segment of corona, s = staminal segment of corona, t = style apex. Methods of specimen preparation and microscopy will be presented elsewhere. —6 (left). *Marsdenia edulis* (*Fishbein 3703*, Sonora, Mexico, WS). The gynostegial corona is composed of free, appressed, deltoid, staminal segments. Scale bar = 1.76 mm. —7 (right). *Asclepias asperula* (material collected by M. Fishbein from the same population as *Martin s.n.*, 10 July 1968, Arizona, U.S.A., ARIZ). The gynostegial corona is composed of large, clavate, deeply grooved, staminal segments united with small interstaminal segments that appear as "m"-shaped platforms. Scale bar = 1.76 mm.

tinct types (i.e., as different from one another as each is from corolline coronas), there are three reasons to prefer treating these types as homologous, differing simply through elaboration in adjacent sectors of the flower. First, interstaminal segments are almost always united with staminal segments (Liede & Kunze, 1993). Second, there are many taxa (e.g., *Cynanchum* L.; Liede & Kunze, 1993; Endress & Bruyns, 2000) in which the corona is tubular, with little elaboration in the staminal or interstaminal sectors. Third, coronas with interstaminal segments, but lacking staminal segments, never occur (Liede & Kunze, 1993). Rather than suggesting that there are two distinct gynostegial corona types, the distribution of corona forms (Kunze, 1990, 1995b, 1997; Liede & Kunze, 1993) implies that homologous gynostegial coronas simply vary in the degree to which elaboration is manifest in the interstaminal sector. The only ontogenetic data bearing on this question (Endress & Bruyns, 2000) are consistent with the interpretation adopted here. Regardless of how these coronas are allocated into types, the evolutionary analysis presented here allows for explicit evaluation of the homology of interstaminal and staminal coronas and the pattern of their diversification.

As with the analysis of corolline coronas, reconstruction of the evolutionary history of gynostegial coronas depends on the assessment of the corona type of Periplocoideae. I begin by assuming that all coronas of Periplocoideae are corolline, as in character 3a, and that gynostegial coronas are absent (character 4a, Tables 2, 3). Under this scenario, the common ancestor of Asclepiadaceae is inferred unambiguously to lack a gynostegial corona (Fig. 8). The time of origin of the gynostegial corona, however, is ambiguous. Asclepiadoideae are inferred to possess a gynostegial corona ancestrally, but the presence of this corona type in the common ancestor of Asclepiadoideae and Secamonoideae is equivocal. It is equally parsimonious to infer that this ancestor lacked a gynostegial corona, possessed a corona of distinct staminal segments, or possessed a corona of united staminal and interstaminal segments. This ambiguity arises because the lineages descending from this ancestor are inferred to have evolved different gynostegial corona types: the ancestral corona type of Asclepiadoideae is composed of staminal and interstaminal segments, but that of Secamonoideae is composed only of staminal segments (Fig. 8). Any step matrix that weights the gain of gynostegial coronas greater (no matter how slightly) than transitions between distinct staminal coronas and united staminal and interstaminal coronas would make the inference of a lack of gynostegial corona in the common ancestor of Asclepiadoideae + Secamonoideae less parsimonious than the presence of either type of gynostegial corona. Thus, weighting the origin of the gynostegial corona greater than transitions between free staminal and united staminal and interstaminal forms supports the single origin of gynostegial coronas in the common ancestor of Asclepiadoideae and Secamonoideae, although the form of the corona is equivocal.

Coronas of united staminal and interstaminal segments are restricted to Asclepiadoideae, and are inferred to be the ancestral corona form in the subfamily (Fig. 8). Although the reduction to two anther locules has long been noted as a synapomorphy of this subfamily (see above), the importance of the form of the gynostegial corona has been less appreciated. Within Asclepiadoideae, there is a low level of homoplasy in gynostegial coronas, among sampled taxa. Most importantly, the tribe Marsdenieae is characterized by a corona composed of distinct staminal segments (Fig. 6). Although this corona type is shared with Secamonoideae, the most parsimonious inference is that it is convergent in these clades, rather than phylogenetically homologous. Thus, a gynostegial corona of distinct staminal segments appears as a synapomorphy of Marsdenieae. It is noteworthy that Swarupanandan et al. (1996) could find no morphological synapomorphy for Marsdenieae, which they submerged under Stapelieae (= Ceropegieae). Convergence of staminal coronas in Marsdenieae and Secamonoideae also refutes Kunze's (1995a) hypothesis that staminal coronas are homologous and plesiomorphic in these groups.

Two instances of homoplasy are indicated in the tribe Asclepiadeae, among sampled taxa. *Cynanchum laeve* (Michx.) Pers. possesses a corona of distinct staminal segments, which must be regarded as convergent with those of Marsdenieae and Secamonoideae. Such coronas are not uncommon among members of subtribe Metastelminae (e.g., *Metastelma* R. Br.). At this time, it is unclear how many such instances of convergence will be demonstrated in Asclepiadeae, because of the limited sampling in the present study. *Cynanchum utahense* (Engelm.) Woodson lacks a corona entirely; this is clearly a reversal to the absence of a gynostegial corona found in Periplocoideae and non-asclepiad Apocynaceae. Other instances of the absence of a gynostegial corona are well known in Asclepiadeae, especially in subtribe Astephaninae (e.g., *Astephanus* R. Br.).

Recoding some Periplocoideae as possessing a gynostegial corona (character 4b, Tables 2, 3) re-

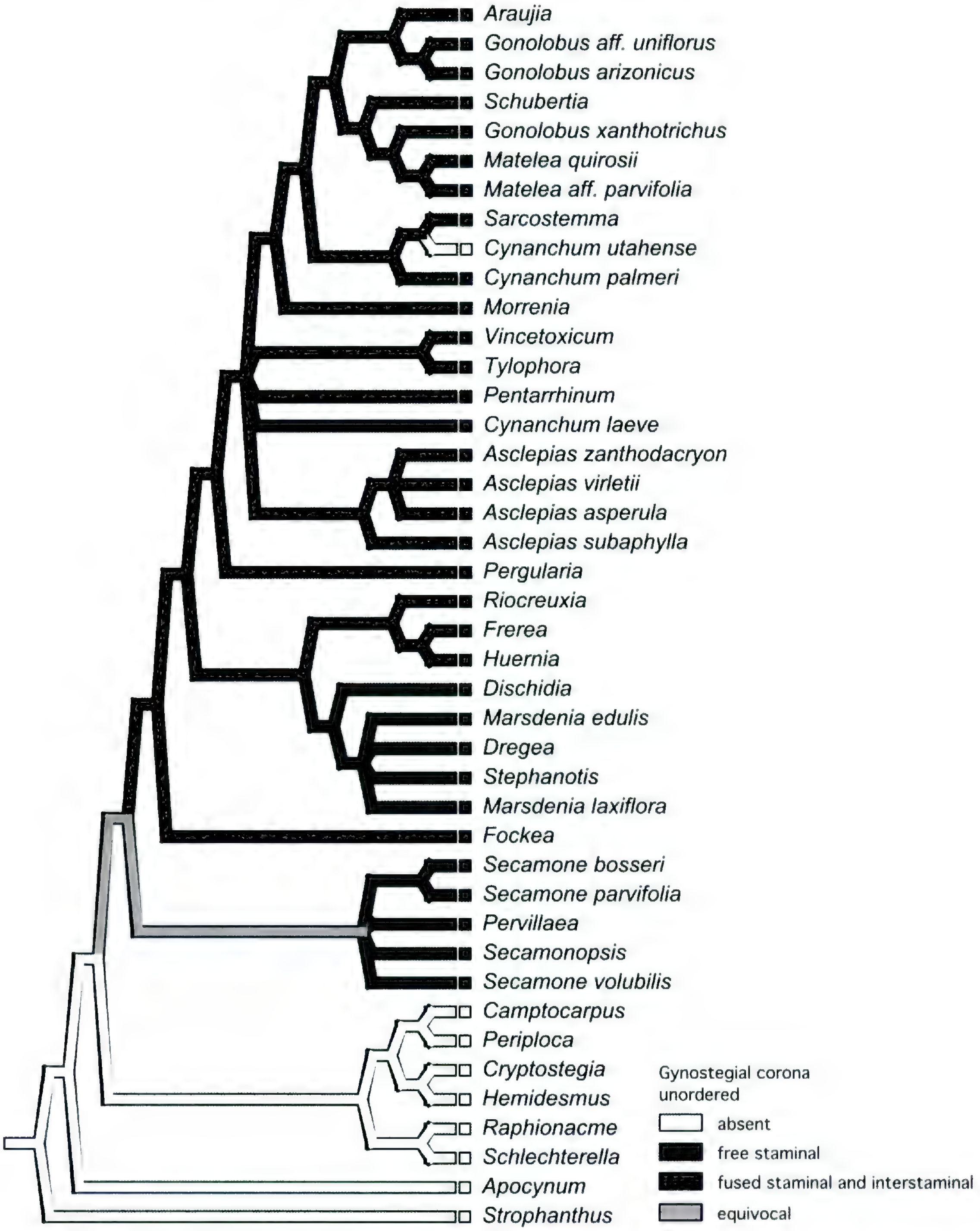


Figure 8. Maximum parsimony reconstruction of the evolutionary history of gynostegial coronas, based on the cladogram in Figure 1 and the coding of character 4a (Tables 2, 3). Character states are treated as unordered with equal transition probabilities among states. The ancestor of and branch leading to Secamonoideae + Asclepiadoideae is reconstructed with equal parsimony to be lacking a gynostegial corona, possessing a corona of free staminal segments, or possessing a corona of united staminal and interstaminal segments. The ancestor of and branch leading to Secamonoideae is reconstructed as equivocal because of the polytomy present in the strict consensus of most-parsimonious trees (MPTs). All resolutions of secamonoid phylogeny present in the MPTs result in reconstruction of the ancestor as possessing a corona of free staminal segments. Additional reconstructions found with alternate transition probabilities are discussed in the text.

sults in ambiguity concerning the ancestral absence of a gynostegial corona in Asclepiadaceae, and introduces additional homoplasy among Periplocoideae (Fig. 9). The presence of gynostegial coronas in some Periplocoideae permits with equal parsimony the common ancestor of Asclepiadaceae to either lack a gynostegial corona or possess one of distinct staminal segments; a corona of united staminal and interstaminal segments, however, is less parsimonious. Similarly, it is equally parsimonious to infer that the ancestor of Periplocoideae lacked a gynostegial corona or possessed one of distinct staminal segments. If such a corona was absent in the ancestor of Periplocoideae, then independent derivation is implied within the subfamily (Fig. 9). If a gynostegial corona was ancestral in Periplocoideae, then one or more losses must have occurred. Because taxa bearing ambiguous corona types may be oversampled in this study (see above), further sampling of Periplocoideae may support the convergent evolution of gynostegial coronas in this subfamily, if they indeed should be coded as gynostegial (Kunze, 1990; Nilsson et al., 1993; Endress & Bruyns, 2000).

PHYLOGENETIC SENSITIVITY

The evolutionary scenarios inferred here from maximum parsimony reconstructions are specific to the preliminary phylogeny derived from analysis of partial *mat*K sequences (L. Civeyrel & M. Fishbein, unpublished data). Errors in the estimation of phylogeny may lead to errors in estimates of timing, location, and degree of homoplasy in character evolution (see Ackerly & Donoghue, 1998, for a robust analysis of phylogenetic sensitivity). An analysis of *rbc*L sequence data suggested that Asclepiadaceae as circumscribed here are polyphyletic (Sennblad & Bremer, 1996). Although support for the polyphyly of Asclepiadaceae found with *rbc*L sequences (Sennblad & Bremer, 1996) was much weaker than support for the monophyly of Asclepiadaceae found with *mat*K (Civeyrel et al., 1998; L. Civeyrel & M. Fishbein, unpublished data), sampling is poor in both data sets and alternate topologies should be considered. A recent study employing a different, non-coding region of chloroplast DNA and broader sampling reported non-monophyly of Asclepiadaceae (Potgieter & Albert, 2001). Thus, I have evaluated most parsimonious reconstructions of the evolution of pollinia and corona characters assuming a closer relationship of Periplocoideae to non-asclepiad Apocynaceae than to the remainder of Asclepiadaceae (Sennblad & Bremer, 1996; Potgieter & Albert, 2001).

The evolutionary reconstructions based on the *mat*K phylogeny are robust to the non-monophyly of Asclepiadaceae. For both pollinia characters, the phylogenetic placement of character transformations is identical for topologies in which Asclepiadaceae are monophyletic or polyphyletic. Because Periplocoideae retain ancestral characteristics of non-asclepiad Apocynaceae, placement of Periplocoideae as sister to these lineages does not alter inferences about character evolution. However, the phylogenetic relationships among unsampled Periplocoideae are unknown, and could support an earlier origin of pollinia in the common ancestor of Asclepiadaceae, if asclepiads are monophyletic. Hypotheses of the pattern of corona evolution are also insensitive to phylogenetic uncertainty, *if* periplocoid coronas are assumed to be uniformly corolline (characters 3a, 4a). However, polyphyly of Asclepiadaceae does affect reconstructions of corona evolution when gynostegial coronas are coded as occurring in some Periplocoideae (characters 3b, 4b). Under this scenario, polyphyly of Asclepiadaceae has no effect on the inference of character evolution in Secamonoideae and Asclepiadoideae. Periplocoideae, however, are unambiguously inferred to ancestrally possess a corolline corona, as in reconstructions of character 3a. As a result, the gynostegial coronas of Periplocoideae are unambiguously inferred to represent convergence with coronas of Asclepiadoideae and Secamonoideae. Thus, polyphyly of Asclepiadaceae does not introduce new hypotheses of character evolution; it merely limits the parsimonious reconstructions of corona evolution under a single scenario, in which some periplocoid coronas are interpreted to be gynostegial.

CONCLUSIONS

The exquisitely complex flowers of Asclepiadaceae are tempting subjects for the study of innovation and diversification. Complexity is due in part to the unusually high degree of structural and functional synorganization between the androecium and gynoecium, as exemplified by the pollinarium. Complexity is due also to elaborate diversification of the corona. Pollinaria and coronas are distinctive features of Asclepiadaceae that potentially represent innovations integral to the radiation of this clade. The phylogenetically explicit approach adopted in this paper provides a preliminary analysis of the timing and patterns of diversification of these structures, as well as an assessment of the level of homoplasy. Although homology assessments and evolutionary reconstructions for these struc-

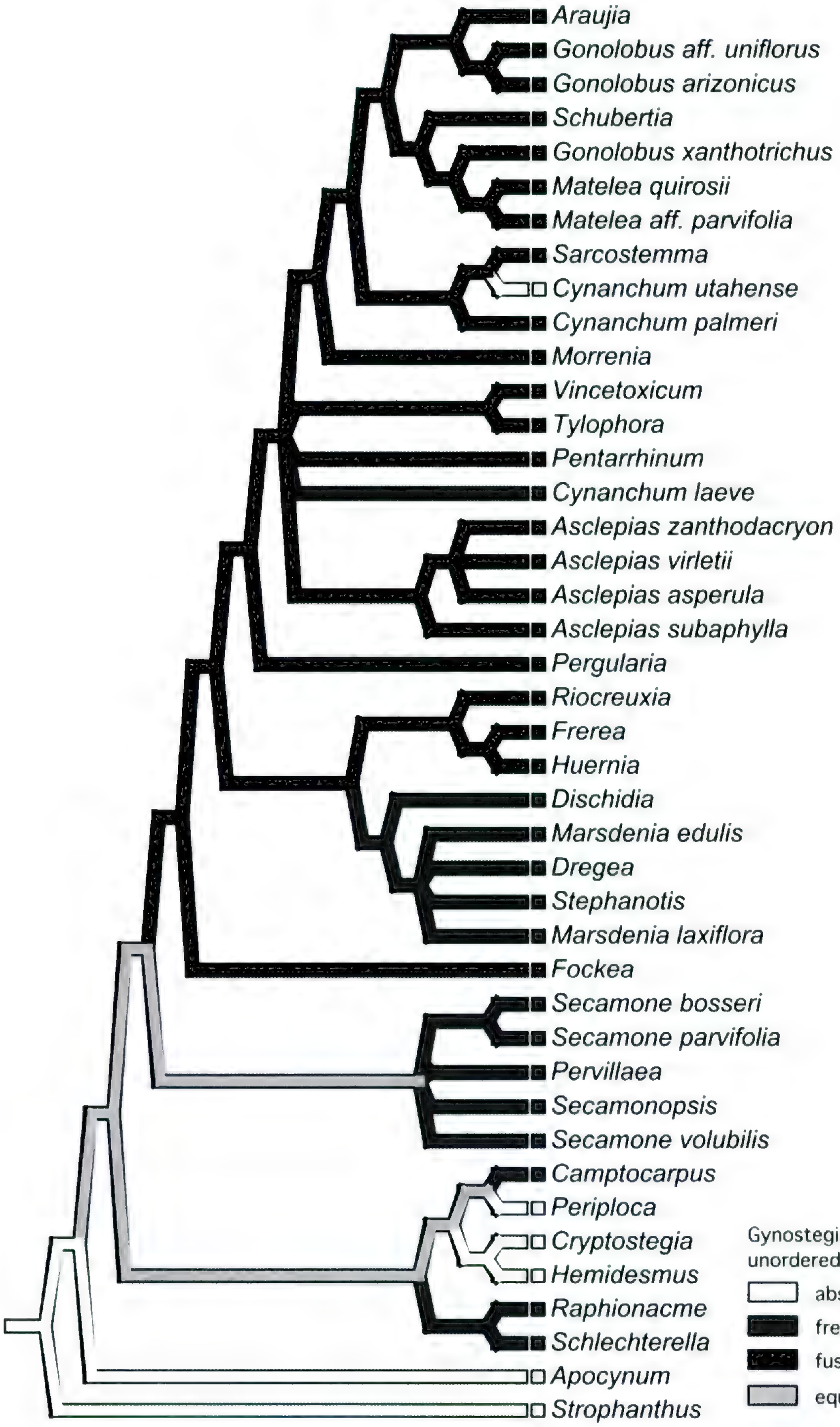


Figure 9. Maximum parsimony reconstruction of the evolutionary history of gynostegial coronas, based on the cladogram in Figure 1 and the coding of character 4b (Tables 2, 3). Character states are treated as unordered with equal transition probabilities among states. The ancestor of and branch leading to Secamonoideae + Asclepiadoideae is reconstructed with equal parsimony to be lacking a gynostegial corona, possessing a corona of free staminal segments, or possessing a corona of united staminal and interstaminal segments. The ancestor of and branch leading to Secamonoideae is reconstructed as equivocal because of the polytomy present in the strict consensus of most-parsimonious trees (MPTs). All resolutions of secamonoid phylogeny present in the MPTs result in reconstruction of the ancestor as possessing a corona of free staminal segments. The ancestor of and branch leading to Asclepiadaceae is reconstructed with equal parsimony to be lacking a gynostegial corona or possessing a corona of free staminal segments; a corona of united staminal and interstaminal segments is less parsimonious.

tures have been presented in the past, rigor has been limited by the lack of an explicit phylogenetic hypothesis (Woodson, 1941; Good, 1956; Kunze, 1990, 1995a; Liede & Kunze, 1993; Swarupanandan et al., 1996; Endress & Bruyns, 2000) or minimal taxon sampling and attention to topological ambiguity and homology decisions (Wanntorp, 1988; Judd et al., 1994). Admittedly, taxon sampling in the present study is still inadequate to fully explore the range of corona evolution of Asclepiadaceae and the origin of pollinia in Periplocoideae, subjects still ripe for further investigation.

Pollinaria and coronas exhibit contrasting evolutionary patterns. The pollinarium is a novel feature of Asclepiadaceae that has undergone relatively conservative evolution with comparatively little diversification. Reconstructions of the evolutionary history of pollinium number per flower and position of translator attachment show little homoplasy. Shifts in the number and orientation of pollinia correspond to the origin of major clades, as largely reflected in classifications of the family (Brown, 1811; Endlicher, 1838; Decaisne, 1844; Schumann, 1895; Liede & Albers, 1994; Liede, 1997; Endress & Bruyns, 2000). Once such changes evolved, no reversals or losses are inferred to have occurred, except for Periplocoideae. Pollinia in this subfamily exhibit additional diversity in the morphology of the constituent pollen grains (Verhoeven & Venter, 2001). More thorough sampling of Periplocoideae in phylogenetic studies is necessary to evaluate the level of convergence and reversal in the evolution of pollinia. Comparative studies that contrast the conservative evolution of pollinia in Secamonoideae and Asclepiadoideae with the greater lability in Periplocoideae may be useful in evaluating Riedl's (1978) concept of evolutionary burden (see also Donoghue, 1989) as it applies to asclepiad flowers.

A further caveat pertains to the scale at which pollinarium evolution is studied. I have discussed the difficulties encountered in prior attempts to describe pollinium orientation (Woodson, 1941; Rosatti, 1989; Kunze, 1995b; Liede, 1996; Swarupanandan et al., 1996; Civeyrel et al., 1998). By adopting homology criteria that are more easily assessed across Asclepiadaceae (Swarupanandan et al., 1996), I have incompletely addressed the extensive variation that led to difficulties in homology assessment in the past. Careful study of variation in pollinium shape and anther orientation in a phylogenetic framework, including much more thorough taxon sampling, is required to assess the lability of pollinarium characteristics not studied in the present investigation.

The evolution of coronas appears to exhibit greater diversification with more extensive homoplasy than that of pollinaria. The presence of corolline coronas is variable in Secamonoideae and, perhaps, Periplocoideae. This variability requires that convergence and/or losses have occurred in the history of corolline coronas. Similarly, evolutionary reconstructions of the presence and form of gynostegial coronas require considerable homoplasy. The inference of evolutionary lability depends largely on homology assessment. Under some scenarios, the origin of subfamilies and tribes may be marked by large changes in corona morphology with no subsequent homoplasy (e.g., the synapomorphic loss of corolline coronas in Asclepiadoideae). In other cases, homoplasy is inferred regardless of homology assessment, as in the convergence and loss of gynostegial coronas of Asclepiadeae. The greater degree of homoplasy in coronas than pollinaria invites inquiry into adaptive explanations. One could speculate that the greater functional diversity of coronas plays an important role. The sole function of pollinaria is the transfer of male gametes. Coronas may participate in the attraction, reward, and positioning of pollinators (Wanntorp, 1974; Kunze, 1991, 1997; Endress, 1994).

Unlike pollinaria, corolline coronas of Asclepiadaceae appear to be plesiomorphic features present in related non-asclepiad Apocynaceae. However, gynostegial coronas are potentially a novel structure of Asclepiadaceae. Gynostegial coronas appear to adopt a novel position in the flower. Different evolutionary scenarios have been proposed (cf. Kunze, 1990), under which (1) gynostegial coronas are a structure *sui generis* of Asclepiadaceae and are not homologous with corolline coronas, or (2) gynostegial coronas are derived from corolline coronas. Evidence for both hypotheses was presented by Kunze (1990), who concluded that gynostegial coronas were non-homologous with corolline coronas. Liede and Kunze (1993) indicated homology among all corona types in a linear transformation series, but also stated that some corona types are not homologous. Circumstantial support for the second scenario comes from the rarity of instances in which corolline coronas and gynostegial coronas are present in the same flower (cf. Figs. 4, 5, 8, 9), although Secamonoideae provide important exceptions (Good, 1956; Safwat, 1962; Kunze, 1990; Klackenberg, 1995; Li et al., 1995; Forster, 1996). Kunze (1990) noted the presence across Asclepiadaceae of a basal synorganized zone during flower development. Endress and Bruyns (2000) proposed the homology of this zone across Asclepiadaceae, interpreting the zone as neither corolline nor sta-

minal and suggesting that at least some corolline and gynostegial coronas may develop from this region of zonal growth. Like homology assessment of corolline and gynostegial coronas, accurate evaluation of the novelty of the gynostegial corona requires ontogenetic study in a phylogenetic framework (Hufford, 1995, 1996a, b). The importance of ontogenetic studies as a basis for homology assessment (see Kaplan, 1984) was recognized in Kunze's (1990) assessment of corona evolution and in the work of Endress and Bruyns (2000). In addition, phylogenetic analysis permits evaluation of phylogenetic homology, assessment of homoplasy, and inference of ontogenetic transformations (Hufford, 1995, 1996a, b). These analytical properties provide the strongest basis for assessing the patterns and mechanisms of morphological evolution and should guide future studies of innovation and diversification of asclepiad flowers.

Literature Cited

Ackerly, D. D. & M. J. Donoghue. 1998. Leaf size, sapling allometry, and Corner's rules: Phylogeny and correlated evolution in maples (*Acer*). Amer. Naturalist 152: 767–791.

Bookman, S. S. 1981. The floral morphology of *Asclepias speciosa* (Asclepiadaceae) in relation to pollination and a clarification in terminology for the genus. Amer. J. Bot. 68: 675–679.

Brown, R. 1811. On the Asclepiadeae, a natural order of plants separated from the Apocyneae of Jussieu. Mem. Wern. Nat. Hist. Soc. 1: 12–78.

Civeyrel, L. 1994. Variation et évolution des types polliniques du genre *Secamone* (Asclepiadaceae, Secamonoideae). Compt. Rend. Hebd. Séances Acad. Sci. 317: 1159–1165.

——— & J. Klackenberg. 1996. A second species of the Madagascan genus *Secamonopsis* (Asclepiadaceae). Novon 6: 144–146.

———, A. Le Thomas, K. Ferguson & M. W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to the molecular phylogeny obtained from plastid *mat*K sequences. Molec. Phylogenet. Evol. 9: 517–527.

Corry, T. H. 1883. On the structure and development of the gynostegium, and the mode of fertilization in *Asclepias cornuti*, Decaisne (*A. syriaca* L.). Trans. Linn. Soc. London, Bot. 2: 173–207.

Decaisne, J. 1844. Asclepiadeae. Pp. 490–665 *in* A. De Candolle (editor), Prodromus Systematis Naturalis, Part 8. Fortin, Masson, Paris.

Donoghue, M. J. 1989. Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. Evolution 43: 1137–1156.

Endlicher, S. L. 1838. Genera Plantarum. Pp. 586–599 *in* Part 8 (Asclepiadeae). Beck, Vienna.

Endress, M. E. & P. V. Bruyns. 2000. A revised classification of Apocynaceae s. l. Bot. Rev. (Lancaster) 66: 1–56.

Endress, P. K. 1994. Diversity and Evolutionary Biology of Tropical Flowers. Cambridge Univ. Press, Cambridge.

Fallen, M. E. 1986. Floral structure in the Apocynaceae: Morphological, functional, and evolutionary aspects. Bot. Jahrb. Syst. 106: 245–286.

Farrell, B. D., D. E. Dussourd & C. Mitter. 1991. Escalation of plant defense: Do latex and resin canals spur diversification? Amer. Naturalist 138: 881–900.

Fishbein, M. & D. L. Venable. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. Ecology 77: 1061–1073.

Forster, P. I. 1996. Asclepiadaceae. Flora of Australia 28: 197–283. [*Hoya*, *Dischidia* by P. I. Forster & D. J. Liddle.] Australian Government Publishing Service, Canberra.

Frye, T. C. 1902. A morphological study of certain Asclepiadaceae. Bot. Gaz. (Crawfordsville) 34: 389–412.

Galil, J. & M. Zeroni. 1965. Nectar system of *Asclepias curassavica*. Bot. Gaz. (Crawfordsville) 126: 144–148.

Good, R. 1956 [1974 reprint]. Features of Evolution in the Flowering Plants. Dover, New York.

Goyder, D. J. 1994. A revision of *Anisopus* N. E. Br. (Asclepiadaceae: Marsdenieae). Kew Bull. 49: 737–747.

Hodges, S. A. 1997. Floral nectar spurs and diversification. Int. J. Pl. Sci. 158 (suppl.): S81–S88.

Hofmann, U. & A. K. Specht. 1986. Der morphologische Charakter der Asclepiadaceencorona. Beitr. Biol. Pflanzen 61: 79–85.

Hooker, J. D. 1883. Asclepiadeae. Pp. 1–78 *in* J. D. Hooker (editor), Flora of British India, Vol. 4. L. Reeve, Kent.

Hufford, L. 1995. Patterns of ontogenetic evolution in perianth diversification of *Besseya* (Scrophulariaceae). Amer. J. Bot. 82: 655–680.

———. 1996a. The morphology and evolution of male reproductive structures of Gnetales. Int. J. Pl. Sci. 157 (suppl.): S95–S112.

———. 1996b. Ontogenetic evolution, clade diversification, and homoplasy. Pp. 271–301 *in* M. J. Sanderson & L. Hufford (editors), Homoplasy: The Recurrence of Similarity in Evolution. Academic Press, San Diego.

Judd, W. S., C. S. Campbell, E. A. Kellogg & P. F. Stevens. 1999. Plant Systematics: A Phylogenetic Approach. Sinauer, Sunderland, Massachusetts.

———, R. W. Sanders & M. J. Donoghue. 1994. Angiosperm family pairs: Preliminary phylogenetic analyses. Harvard Pap. Bot. 5: 1–51.

Kaplan, D. R. 1984. The concept of homology and its central role in the elucidation of plant systematic relationships. Pp. 51–70 *in* T. Duncan & T. F. Stuessy (editors), Cladistics: Perspectives on the Reconstruction of Evolutionary History. Columbia Univ. Press, New York.

Klackenberg, J. 1992a. Taxonomy of *Secamone* s. lat. (Asclepiadaceae) in the Madagascar region. Opera Bot. 112: 1–127.

———. 1992b. Taxonomy of *Secamone* (Asclepiadaceae) in Asia and Australia. Kew Bull. 47: 595–612.

———. 1995. Revision of the Malagasy genus *Pervillea* (Asclepiadaceae) and its phylogenetic relationship to *Calyptranthera*. Nordic J. Bot. 16: 165–184.

———. 1997. Revision of the genus *Baroniella* Costantin & Gallaud (Asclepiadaceae, Periplocoideae). Candollea 52: 383–407.

———. 1998. Taxonomy and phylogeny of the genus *Camptocarpus* s.l. (Periplocoideae, Asclepiadaceae). Bot. Jahrb. Syst. 120: 45–85.

Kunze, H. 1990. Morphology and evolution of the corona in Asclepiadaceae and related families. Trop. Subtrop. Pflanzenwelt 76: 1–51.

———. 1991 Structure and function in asclepiad pollination. Pl. Syst. Evol. 176: 227–253.
———. 1993. Evolution of the translator in Periplocaceae and Asclepiadaceae. Pl. Syst. Evol. 185: 99–122.
———. 1995a. Bau und Funktion der Asclepiadaceenblüte. Phyton (Horn) 35: 1–24.
———. 1995b. Floral morphology of some Gonolobeae (Asclepiadaceae). Bot. Jahrb. Syst. 117: 211–238.
———. 1997. Corona and nectar system in Asclepiadinae (Asclepiadaceae). Flora 192: 175–183.
Li, P.-T., M. G. Gilbert & W. D. Stevens. 1995. Asclepiadaceae. Pp. 189–270 *in* Z.-Y. Wu & P. H. Raven (editors), Flora of China, Vol. 16. Science Press, Beijing, and Missouri Botanical Garden, St. Louis.
Liede, S. 1996. *Cynanchum–Rhodostegiella–Vincetoxicum–Tylophora* (Asclepiadaceae): New considerations on an old problem. Taxon 45: 193–211.
———. 1997. Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae): A synopsis. Taxon 46: 233–247.
———. 2001. Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) reconsidered: New evidence based on cpDNA spacers. Ann. Missouri Bot. Gard. 88: 657–668.
——— & F. Albers. 1994. Tribal disposition of genera in the Asclepiadaceae. Taxon 43: 201–231.
——— & H. Kunze. 1993. A descriptive system for corona analysis in Asclepiadaceae and Periplocaceae. Pl. Syst. Evol. 185: 275–284.
Liem, K. F. 1974. Evolutionary strategies and morphological innovations: Cichlid pharyngeal jaws. Syst. Zool. 22: 425–441.
Maddison, W. P. & D. R. Maddison. 1992. MacClade: Analysis of Phylogeny and Character Evolution, vers. 3.05. Sinauer, Sunderland, Massachusetts.
Mayr, E. 1960. The emergence of evolutionary novelties. Pp. 349–380 *in* S. Tax (editor), The Evolution of Life. Univ. Chicago Press, Chicago.
Mitter, C., B. Farrell & B. Wiegmann. 1988. The phylogenetic study of adaptive zones: Has phytophagy promoted insect diversification? Amer. Naturalist 132: 107–128.
Nilsson, S., M. E. Endress & E. Grafström. 1993. On the relationship of the Apocynaceae and Periplocaceae. Grana Suppl. 2: 3–20.
Nitecki, M. H. (Editor). 1990. Evolutionary Innovations. Univ. Chicago Press, Chicago.
Patterson, C. 1982. Morphological characters and homology. Pp. 21–74 *in* K. A. Joysey & A. E. Friday (editors), Problems of Phylogenetic Reconstruction. Academic Press, London.
Potgieter, K. & V. A. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.
Riedl, R. 1978. Order in Living Organisms. Wiley, New York.
Rosatti, T. J. 1989. The genera of suborder Apocynineae (Apocynaceae and Asclepiadaceae) in the southeastern United States. Asclepiadaceae. J. Arnold Arbor. 70: 443–514.
Safwat, F. M. 1962. The floral morphology of *Secamone* and the evolution of the pollinating apparatus in Asclepiadaceae. Ann. Missouri Bot. Gard. 49: 95–129.
Schill, R. & U. Jäkel. 1978. Beitrag zur Kenntnis der Asclepiadaceen-Pollinarien. Trop. Subtrop. Pflanzenwelt 22: 1–122.
Schumann, K. 1895. Asclepiadaceae. Pp. 189–306 *in* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien, Vol. IV(2). Wilhelm Engelmann, Leipzig.
Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.
Sundell, E. 1981. The New World species of *Cynanchum* L. subgenus *Mellichampia* (A. Gray ex Wats.) Woods. (Asclepiadaceae). Evol. Monogr. 5: 1–63.
Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. C. Basha. 1996. The subfamilial and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.
Venter, H. J. T. & R. L. Verhoeven. 1997. A tribal classification of the Periplocoideae (Apocynaceae). Taxon 46: 705–720.
——— & ———. 1998. A taxonomic revision of *Schlechterella* (Periplocoideae, Apocynaceae). S. African J. Bot. 64: 350–355.
——— & ———. 2001. Diversity and relationships within the Periplocoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 550–568.
Verhoeven, R. L. & H. J. T. Venter. 1994. Pollen morphology of the Periplocaceae from Madagascar. Grana 33: 295–308.
——— & ———. 1997. The translator of *Raphionacme* (Periplocaceae). S. African J. Bot. 63: 46–54.
——— & ———. 1998a. Pollinium in *Schlechterella*: Periplocoideae (Apocynaceae). S. African J. Bot. 64: 256–257.
——— & ———. 1998b. Pollinium structure in Periplocoideae (Apocynaceae). Grana 37: 1–14.
——— & ———. 2001. Pollen morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). Ann. Missouri Bot. Gard. 88: 569–582.
Wanntorp, H.-E. 1974. *Calotropis gigantea* (Asclepiadaceae) and *Xylocopa tenuiscapa* (Hymenoptera, Apidae). Studies in flower morphology and pollination biology. Svensk Bot. Tidskr. 68: 25–32.
———. 1988. The genus *Microloma* (Asclepiadaceae). Opera Bot. 98: 1–69.
Woodson, R. E., Jr. 1941. The North American Asclepiadaceae I. Perspective of the genera. Ann. Missouri Bot. Gard. 28: 193–244.

A KARYOLOGICAL SURVEY OF ASCLEPIADOIDEAE, PERIPLOCOIDEAE, AND SECAMONOIDEAE, AND EVOLUTIONARY CONSIDERATIONS WITHIN APOCYNACEAE S.L.[1]

Focke Albers[2] and Ulrich Meve[3]

Abstract

The chromosome numbers of 672 species out of 740 taxa of the Apocynaceae in the subfamilies Asclepiadoideae, Periplocoideae, and Secamonoideae were either determined or extracted from the literature. The chromosome numbers of 299 taxa are published here for the first time, and a further 139 new counts confirm or complete previously published chromosome numbers. The basic chromosome number $x = 11$ is found to be predominant, occurring in ca. 96% of the taxa investigated. Deviations from $x = 11$ are absent in Periplocoideae and Secamonoideae, but some variation is present in Asclepiadoideae. A reduction of the karyotype to $x = 10$ is known only in the advanced tribe Asclepiadeae, where it is important in the Old World subtribe Astephaninae (*Microloma*), as well as in the New World subtribes Metastelminae (*Funastrum*, *Orthosia*, *Philibertia*, and *Grisebachiella*) and Oxypetalinae (*Araujia* and *Tweedia*). An increase ($x = 12, 13, 14$) was found only rarely and sporadically in the subfamily Asclepiadoideae. About 6% of the species (7% of the taxa) investigated are found to be polyploid. The majority of such taxa are tetraploid with $2n = 44$; only a few are hexaploid or higher. The data on chromosome number and size presented in this paper are interpreted in relation to the systematic position and geographical distribution of the taxa investigated. On the average karyotype size diminishes from rather large chromosomes in the Periplocoideae to the smallest karyotype length in the presumed most advanced tribe of the Asclepidoideae, the Asclepiadeae. Reduction in the basic chromosome number is regarded as only a rather recent step in genome evolution within Asclepiadeae, which has been taken place in parallel in the Old and New Worlds. Finally, comparision with data available for the Apocynaceae s. str. (Apocynoideae and Rauvolfioideae) is presented.

Key words: Apocynaceae, Asclepiadoideae, chromosome number, chromosome size, evolution, Periplocoideae, polyploidy, Secamonoideae.

According to the most recent classification, the Apocynaceae comprise the subfamilies Apocynoideae, Rauvolfioideae, Periplocoideae, Secamonoideae, and the Asclepiadoideae (Endress & Bruyns, 2000). Of these, the Periplocoideae (cf. Schlechter, 1905; Venter, 1997) and the Asclepiadoideae (Brown, 1810; Bruyns & Forster, 1991) have often been treated as separate families Periplocaceae Schltr. and Asclepiadaceae R. Br. in the past. However, present molecular data support earlier morphological insights that the most natural classification is to treat the group as a single family, and that the subfamily Asclepiadoideae is the most highly evolved group being nested within Apocynaceae s. str., and thus must be included in Apocynaceae for the family to be monophyletic (Olmstead et al., 1993; Sennblad & Bremer, 1996). Approximately 400 genera of Apocynaceae contain about 4300 species. All subfamilies except the small Secamonoideae (9 genera, ca. 170 species; Klackenberg, in press) have been split into tribes and subtribes established mainly on morphological reasoning. Periplocoideae, which is characterized by several monotypic genera, comprises only ca. 180 species distributed among 31 genera (Venter & Verhoeven, 1997, 2001 this volume). Asclepia-

[1] We are very much indebted to the many people and institutions that provided us with plant material over the years, in particular, Jerry Barad (Flemington, NJ), Peter Bruyns (Cape Town, RSA), Karoo Botanic Garden (Worcester, RSA), John Lavranos (Loule, Portugal), Sigrid Liede (Bayreuth, FRG), Inge Pehlemann (Windhoek, Namibia), and Darrel Plowes (Mutare, Zimbabwe). Simone Engel, Mathilde Frönd, Ralf Huesmann, and Björn Niggemann (all Münster) contributed with chromosome measurements and technical assistance to parts of the work. The efforts in improving our manuscript made by two anonymous referees and Victoria Hollowell (St. Louis, MO), but especially by the first editor of this volume, Mary Endress (Zürich, Switzerland), are gratefully acknowledged. This long-term study was funded by the Deutsche Forschungsgemeinschaft (AL 218/1-1, 218/2-1).

[2] Institut für Botanik, Westf. Wilhelms-Universität Münster, Schlossgarten 3, 48149 Münster, Germany.

[3] Lehrstuhl für Pflanzensystematik, Universität Bayreuth, 95440 Bayreuth, Germany.

doideae encompasses ca. 190 genera (for more details see: "Tribal disposition of Periplocoideae and Asclepiadoideae genera," <http://www.uni-bayreuth.de/departments/planta2/>) and more than 2500 species. Relationships within this subfamily are most accurately represented by four tribes: the basal Fockeeae, the Marsdenieae, the Ceropegieae Orb. (this name replaces the up to recently used but younger name Stapelieae Decne.; cf. Endress & Bruyns, 2000), and the Asclepiadeae, which probably is the most advanced. The latter is subdivided into seven subtribes (sensu Liede, 1997, 2001 this volume).

Many studies have demonstrated the taxonomic value of chromosomal information in the plant kingdom in general (Stebbins, 1971; Lewis, 1980). In Asclepiadoideae, however, due to the dominance of $2n = 22$ and a "small" chromosome size, chromosomal data have been little used. Only rarely has karyology, in particular polypoidy, been utilized to support taxonomic conclusions or phylogenetic considerations (Meve, 1995a, 1997a). The study presented here is the result of a long-term project, which was initiated by the late G. Reese (Kiel), with chromosome counts for stapeliads (Reese, 1971; Reese & Kressel, 1967) and continued by us. A summary of the previous literature and a first overview were given by Albers (1983), and many of the further counts have been published in Albers (1974, 1975, 1976, 1977, 1981), Albers and Delfs (1983), Albers and Austmann (1987), and Albers et al. (1988, 1990). In the last 20 years we have emphasized karyotype length investigations in addition to the mere chromosome counts, and with the standard use of well-documented voucher material. We expanded the study to all tribes of the Asclepiadoideae (cf. Albers, 1983; Albers et al., 1993; Meve, 1999), as well as to Periplocoideae and Secamonoideae (Appendix 1). In addition, research included measurements of the DNA content (Huesmann, 1993).

Material and Methods

materials

The data gathered and used in this paper regarding Periplocoideae, Secamonoideae, and Asclepiadoideae are based mainly on our own investigations. Appendix 1 summarizes our recent findings and includes two types of data: first chromosome counts for taxa, which are unmarked, and counts confirming previous chromosome numbers, which are preceded by a hatch symbol (#). Where the same chromosome number was counted for different geographic regions, sometimes two different vouchers of a single taxon are given here. In addition, vouchers for chromosome measurements that are based on plants with already published chromosome numbers are included in Appendix 1. This material is marked by an asterisk (*). For the different calculations, we considered literature data as far as possible, except for information that was either unreliable or doubtful (e.g., for invalid and dubious taxa, presumably wrong counts, etc.). Chromosome numbers published without voucher provenance have been excluded as well, since the chance of including numbers being attributed to incorrectly determined plants increases considerably using undocumented material.

Information on Apocynoideae and Rauvolfioideae is mainly based on Van der Laan and Arends (1985). Information on Apocynaceae s.l. is supplemented by data extracted from Darlington and Wylie (1955), Fedorov (1974), and various volumes of the *Index to Plant Chromosome Numbers* edited by Ornduff (1967), Moore (1973, 1974, 1977), Goldblatt (1981, 1984, 1985, 1988), and Goldblatt and Johnson (1990, 1991, 1994, 1996, 1998).

methods

Chromosome numbers and chromosome sizes were established from adventitious root tip squash preparations. Ten mitotic metaphase plates from different root tips of each sample were investigated, though ten plates were not available in ca. one third of the samples due to the lack of actively growing root tips. The root tips were prepared as follows: pre-treatment in 0.002 M hydroxyquinoline for 4 hr. at 20°C (Tjio & Levan, 1950); fixation in Carnoy's solution for 24 hr. at 20°C; staining in carmine for 24 hr. at 60°C (Snow, 1963). For cytological investigation a Leitz Orthoplan microscope was used. The total lengths of all chromsomes in the genome were measured on maximally contracted chromosomes on the basis of camera lucida pencil drawings. Control (digital) measurements of the karyotype length were done by using the Leitz Orthoplan microscope in connection with a Leica Quantiment 500 (software QWIN). Measurements of the mean value, standard deviation, and error were part of the Leica program.

For fixations of Feulgen cytophotometric measurements of relative 2C DNA content Carnoy's solution was used (24 hr., 4°C) after pretreatment with 0.002M hydroxyquinoline for 4 hr.; ten fixed root tips from each taxon were hydrolyzed in 5M HCl for 80 min. at 20°C, washed in distilled water for 10 min., and stained in Feulgen reagent for 80 min. at room temperature. The washed root tips were

Table 1. Number and distribution of karyologically known species, subspecies, and varieties in Periplocoideae, Secamonoideae, and Asclepiadoideae (number of karyologically known genera in parentheses following the tribal names).

	PERIPLOCO-IDEAE	SECAMONO-IDEAE	ASCLEPIADOIDEAE			
Chromosome number	— (13)	Secamoneae (1)	Fockeeae (2)	Marsdenieae (7)	Ceropegieae (37)	Asclepiadeae (41)
$2n = 18$						3
$2n = 20$						15
$2n = 22$	27	3	6	29	464	134
$2n = 24$					1	2
$2n = 26$						1
$2n = 28$						1
$2n = 40$						1
$2n = 44$	4	1			36	4
$2n = 66$					5	1
$2n =$ ca. 132					1	
$2n =$ ca. 154						1

incubated in 45% acetic acid for 10 min. Then only the dark-stained tips were squashed and the telophases (2C DNA content) were studied with the aid of a Zeiss scanning microscope photometer (SMP 05) using the two wavelength method published by Ornstein (1952) and Patau (1952), and the wavelengths λ max. = 575 nm and λ/2 = 510 nm as published by Greilhuber (1986). The DNA values were obtained on the basis of optical density. This was converted to pg according to Patau (1952). *Allium cepa* (2C DNA content = 33.5 pg [Bennett & Smith, 1976]) was taken as standard.

Results

Chromosome numbers

Karyological information for 672 species, representing ca. 24%, of the three subfamilies Asclepiadoideae, Periplocoideae, and Secamonoideae in 101 genera, representing ca. 44%, and belonging to 740 taxa altogether is summarized in Table 1. The chromosome numbers of 299 taxa are new to science and appear with locality data and vouchers in the compilation of Appendix 1 (unmarked), together with new counts confirming or completing earlier counts (preceded by #). For all counts, also including previously published chromosome numbers of both our and foreign counts, consult the expanded version of Appendix 1 under <http://www.uni-muenster.de/Biologie/botanik/AgAlbers.htm> or <http://www.uni-bayreuth.de/departments/planta2/>.

The basic chromosome number $x = 11$ is by far the most predominant number in Asclepiadoideae, Periplocoideae, and Secamonoideae (Table 1; Fig. 2); only ca. 3.5% of the studied species and taxa deviate from this. Among the deviant base numbers $x = 10$ (Fig. 3C) is the most frequent (ca. 2.5% of the studied species), followed by $x = 9$ (ca. 0.5% of the studied species), and the sporadic increasing deviates $x = 12$, 13, and 14 (Table 1).

In our analysis of the distribution of the basic chromosome number $x = 11$ in the asclepiad subfamilies, tribes, and subtribes neither the Periplocoideae nor the Secamonoideae contained any deviating numbers (Table 1, Fig. 2, Appendix 1). The same situation is found in the basal Asclepiadoideae tribes Fockeeae and Marsdenieae, the large Ceropegieae, and also in Asclepiadeae in the subtribes Gonolobineae, Asclepiadinae, and Tylophorinae (Table 1, Fig. 2, Appendix 1). All known deviating numbers are scattered among the three Asclepiadeae subtribes Astephaninae, Metastelminae, and Oxypetalineae (Table 1, Appendix 1). No taxonomic subunit above generic level is characterized by a basic number deviating from $x = 11$, but single genera like *Microloma* (Fig. 3C) and *Funastrum*, except *F. crispum* (cf. Appendix 1), share exclusively $x = 10$ chromosomes (Albers et al., 1993).

Polyploidy

In the Periplocoideae and Secamonoideae polyploidy occurs only as autoploidy, as simple doubling of $2n = 22$ (Table 1). These genomes with $2n = 44$ are only known from a few *Raphionacme* species (Fig. 2A), *Periploca visciformis*, and from one individual of *Secamone parviflora* (Appendix 1).

Approximately 6% of the species in the Asclepiadoideae are polyploids (Table 1). Polyploidy is

known to occur in 17 genera and is based on $x = 11$, except for the single determination of $2n = 40$ for *Tweedia brunonis* (Asclepiadeae, Oxypetalinae, Appendix 1). Some 85% of these polyploids are tetraploid ($2n = 44$) and 12% are hexaploid ($2n = 66$). The remaining instances of polyploidy include dodecaploidy ($2n = 132$) in the stapeliad *Caralluma burchardii* subsp. *burchardii* (Ceropegieae) (Meve, 1995a) and decatetraploidy in the twiner *Tylophora anomala* N. E. Br. (Asclepiadeae, Tylophorinae) (Meve, 1999). The latter taxon also includes the highest number ever found, $2n = 154$, in an isolated cytotype on Mt. Cameroon, whereas the frequent East African representative of this species is hexaploid with $2n = 66$ (Meve, 1999). The majority of polyploids are present in the tribe Ceropegieae, where 9.3% of the species (8.3% of the taxa) were found to be polyploid (Table 1, Fig. 3B). Stem-succulent stapeliad genera like *Duvalia*, *Orbea*, and *Stapelia* have especially high percentages of polyploid taxa (cf. Albers & Meve, 1991). The genus *Duvalia* in southern Africa, in particular, is a classic example of a polyploid complex including several tetraploids and two hexaploids, and contributes considerably to the understanding of phylogeny and biogeography of *Duvalia* and the African stapeliads in general since polyploidy is a uni-directional event (Meve, 1997a).

Infraspecific polyploidy is comparatively rare in the groups investigated. We did not find it in Periplocoideae, and only once in Secamonoideae (*Secamone parviflora*, Appendix 1). In Asclepiadoideae, especially Ceropegieae, it is less rare, but can be restricted to individuals (cf. *Huernia keniensis*, Appendix 1). Also, mixoploid individuals occur with single sections of the root tip meristem being polyploid instead of diploid (e.g., *Caralluma adscendens* var. *gracilis*: Fig. 3B, Appendix 1; cf. also Albers & Meve, 1991). In some cases the different levels characterize infraspecific taxa, e.g., in *Caralluma burchardii* (Meve, 1995a), *Duvalia sulcata* (Meve, 1997a), *Ceropegia racemosa*, and *Orbeopsis gerstneri* (Appendix 1), and in *Sarcostemma viminale*, where the subspecies *thunbergii* is a tetraploid (Liede & Meve, 1993) while the other seven subspecies are all diploid (Liede & Meve, 1995; Meve & Liede, 1996; Appendix 1).

CHROMOSOME MORPHOLOGY

Chromosomes of the groups under discussion are morphologically rather homogeneous. Chromosomes are typically (sub)metacentric, rarely acrocentric, and their karyograms offer very little variation. One pair of chromosomes per genome possessing secondary constrictions with satellites is the rule as shown in Figure 2A for *Raphionacme hirsuta*, and in Albers (1983: fig. 2a, p. 797) for *Brachystelma dinteri* Schltr. Heterochromatic regions, which have an affinity for Giemsa C-banding stain are consistently located on both sides of the centromere (Albers, 1983: fig. 2b, c) and are therefore of poor taxonomic value. However, the size of chromosomes varies considerably throughout the subfamilies and tribes studied (Figs. 1 and 2).

Single chromosome length varies from ca. 0.6 μm (sometimes less) to 1.7 μm (in rare cases more than 2.0 μm) comparing the tribes and subfamilies (Fig. 1). Within a single karyotype the chromosomes are comparatively similar in size. Only rarely were heterogeneous karyotypes found where chromosome sizes varied considerably (e.g., *Microloma incanum*: min. 0.68—max. 1.12 μm, Fig. 3C; *Heterostemma herberti*: min. 0.8—max. 1.4 μm, Meve, unpublished). In other cases, just one pair of chromosomes is ca. 30% larger than all the remaining, similarly smaller chromosomes (e.g., *Glossonema boveanum*, *Pentarrhinum insipidum*) (Appendix 1). The chromosomes of polyploid taxa are usually smaller than those of diploid ones. This behavior is repeated in infraspecific polyploid units, individuals, or mixoploid tissue.

Some general tendencies at subfamilial, tribal, and subtribal level can be recognized. However, it should be kept in mind that sample sizes vary considerably. This is so because most species are rarely collected at all, are difficult to transplant from the wild, and set seed only sporadically. Only species with succulent stems (stapeliads), tubers (e.g., *Raphionacme*), etc., are well represented in collections, whereas the majority of species in all three subfamilies are lianas or herbaceous vines without storage organs. Therefore, access to living material, especially taxa of the subfamily Periplocoideae and Secamonoideae (but also New World taxa), is restricted, and data concerning their chromosomes are in part rather limited despite all efforts made to obtain as much material as possible. As far as known, the average chromosome length in Periplocoideae differs between various genera. Whereas in *Periploca*, *Petopentia*, and *Stomatostemma* chromosomes are on the average about 1 μm long, those of *Raphionacme*, *Taccazea*, and *Schlechterella* (incl. *Triodoglossum*) are strikingly larger (ca. 1.5 μm; Fig. 1). The few chromosome sizes known for secamonoid taxa reach on the average 1.25 μm in length. This size is similar to that in some Periplocoideae. On the average, however, secamonoid chromosomes are slightly smaller (Fig. 1).

The large subfamily Asclepiadoideae comprises

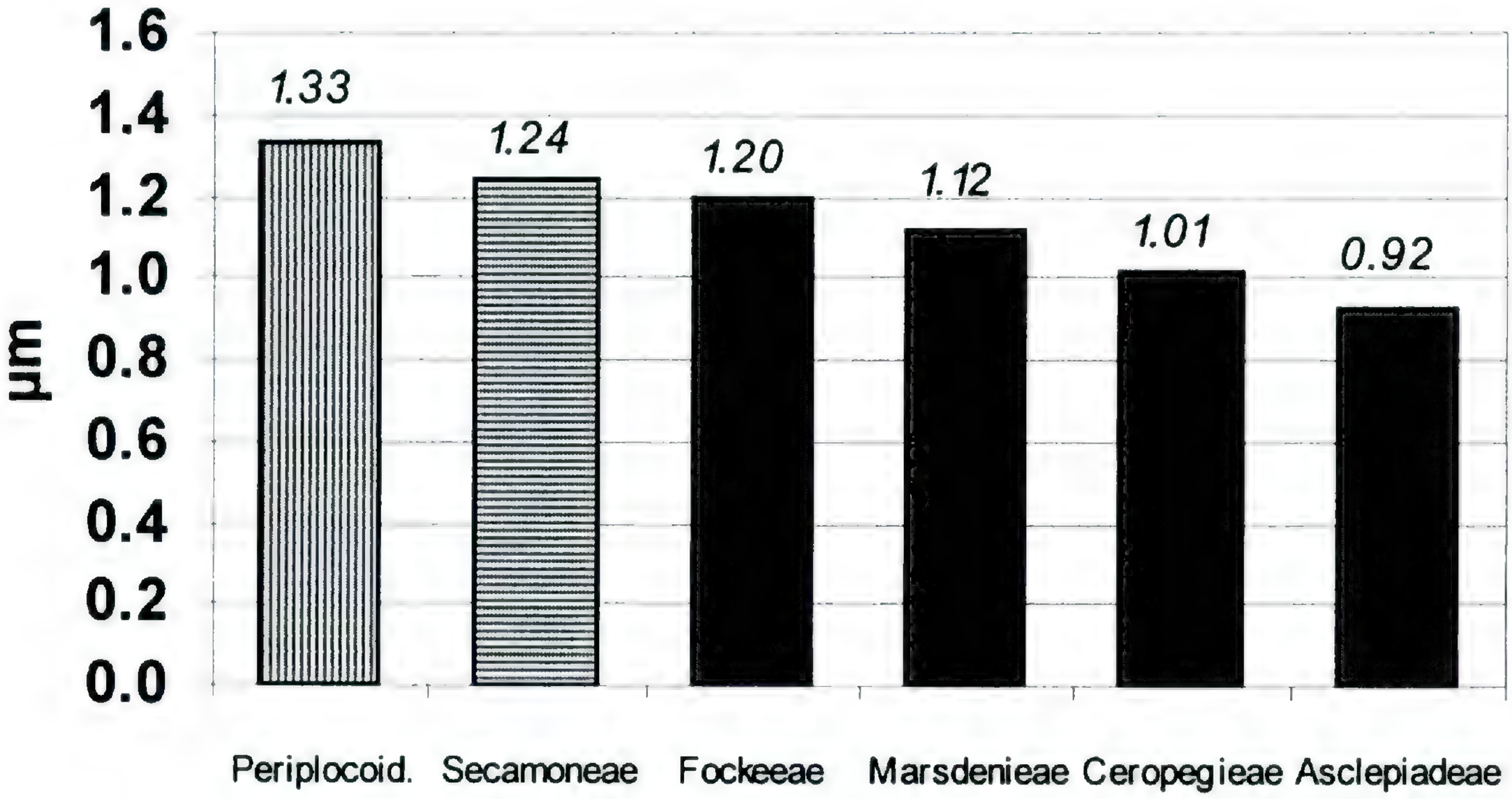


Figure 1. Average chromosome lengths in subfamily Periplocoideae (vertically striped column), Secamonoideae (horizontally striped column), and Asclepiadoideae (gray columns) with the four tribes Fockeeae, Marsdenieae, Ceropegieae, and Asclepiadeae. The ranges are in Periplocoideae: $\bar{x}$ (min. 1.06 µm [*Stomatostemma*]—max. 1.61 µm [*Raphionacme*]), number of samples: 12; Secamonoideae: $\bar{x}$ (min. 1.13 µm—max. 1.34 µm), number of samples: 3 *Secamone*; Fockeeae: $\bar{x}$ (min. 1.15 µm [*Cibirhiza*]—max. 1.27 µm [*Fockea*]), number of samples: 3; Marsdenieae: $\bar{x}$ (min. 0.91 µm [*Hoya*]—max. 1.44 µm [*Telosma*]), number of samples: 17; Ceropegieae: $\bar{x}$ (min. 0.68 µm [*Duvaliandra*]—max. 1.72 µm [*Neoschumannia*]), number of samples: 176; Asclepiadeae: $\bar{x}$ (min. 0.74 µm [*Philibertia*]—max. 1.15 µm [*Gomphocarpus*]), number of samples: 55.

the four tribes Fockeeae, Marsdenieae, Ceropegieae, and Asclepiadeae. The Gonolobeae, which were earlier considered to be a tribe of their own, were transferred by Liede (1997) as subtribe Gonolobinae to the Asclepiadeae. The chromosomes of the Fockeeae have an average length of 1.20 µm, which is not much shorter than in the Secamonoideae (1.24 µm, Fig. 1). The chromosome lengths in the remaining tribes are on average smaller, being 1.12 µm in Marsdenieae, 1.01 µm in Ceropegieae, and 0.92 µm in Asclepiadeae (Fig. 1). The situation in the tribe Marsdenieae is similar to the two groups presented above: parts of the genera *Telosma* and *Marsdenia* have rather large chromosomes (ca. 1.4 µm on the average, but just 1.12 µm in the depicted *Telosma accedens*, Fig. 2D), while all other marsdeniads investigated have smaller chromosomes around 1 µm. The best studied group is the tribe Ceropegieae, where the stem-succulent representatives such as *Caralluma*, *Huernia*, *Orbea*, *Stapelia*, etc., the "stapeliads," represent the main stock of species (ca. 350), followed by *Ceropegia* (180) and *Brachystelma* (ca. 100 species). Almost 90% of the stapeliad species and infraspecific taxa are karyologically known, the best characterized group in the family. The smaller genera such as *Hoodia*, *Stapelianthus*, or *Tridentea* are usually rather homogeneous as to chromosome size, but as the size of the genera increases, variation proportionally increases in some genera. For example, in the large genus *Ceropegia*, the average chromosome length varies between 0.8 µm (*C. occidentalis*) and ca. 1.5 µm (*C. rupicola*; Meve & Albers, unpublished). A similiar pattern is found in *Brachystelma* with averages of 0.78 µm in *B. filifolia* and 1.48 µm in *B. burchellii* (Meve & Liede, 2001a). Chromosomes in most species and genera of the Ceropegieae, however, have an average length of around 1 µm (Fig. 1). Noteworthy outliers at the lower end are the genera *Duvaliandra* and *Leptadenia*. In the monotypic stapeliad genus *Duvaliandra* conspicuously small chromosomes (0.68 µm) were found. In *Leptadenia* (two species investigated, cf. Appendix 1), the average length is 0.72 µm (Meve & Albers, unpublished). Relatively large chromosome sizes are found in species expressing evolutionarily basic morphological characters (*Caralluma edulis*, *C. sinaica* [1.40 µm, Huesmann, 1993], *Ceropegia sankuruensis* [1.37 µm; Meve, unpublished]). The largest chromosomes of the whole tribe (1.72 µm on the average) were observed in *Neoschumannia kamerunensis* (Meve, 1997b). This strong twiner is isolated from all other species of the tribe by its 3-seriate corona. Meve (1995b) considered *Neoschumannia* to be primitive in the Ceropegieae based on morphological characters. With-

in the Ceropegieae, genera with a twining, non-succulent habit, such as *Heterostemma*, *Leptadenia*, or *Orthanthera*, form a heterogeneous assemblage around the stapeliads and their relatives such as *Brachystelma* and *Ceropegia*. In contrast to *Ceropegia* and the stapeliads, chromosomes in *Heterostemma*, *Leptadenia*, and *Orthanthera* are on average ca. 0.25 μm smaller.

The smallest chromosomes, however, are found in the Asclepiadeae (Fig. 1), in particular the subtribes Asclepiadinae, Astephaninae, Metastelminae, and Oxypetalinae (ranging from 0.7 to 1.15 μm on the average). Usually, their chromosomes are less than 1 μm long. Only in one subtribe of the Asclepiadeae, the Gonolobinae, are chromosomes on the average larger than 1.05 μm.

Comparing average chromosome lengths in the three subfamilies as well as those for the tribes of the Asclepiadoideae, a general tendency of size reduction can be seen (Figs. 1, 2). Stepwise, starting with the presumably most primitive subfamily Periplocoideae to the higher evolved Asclepiadoideae, and within the latter subfamily starting with the most primitive Fockeeae to the most advanced Asclepiadeae, a decrease in chromosome size has taken place (Fig. 1). Chromosome size evolution recurs in a similar pattern within lower units such as subtribes or groups of related genera as such as the stapeliads.

2C DNA VALUES

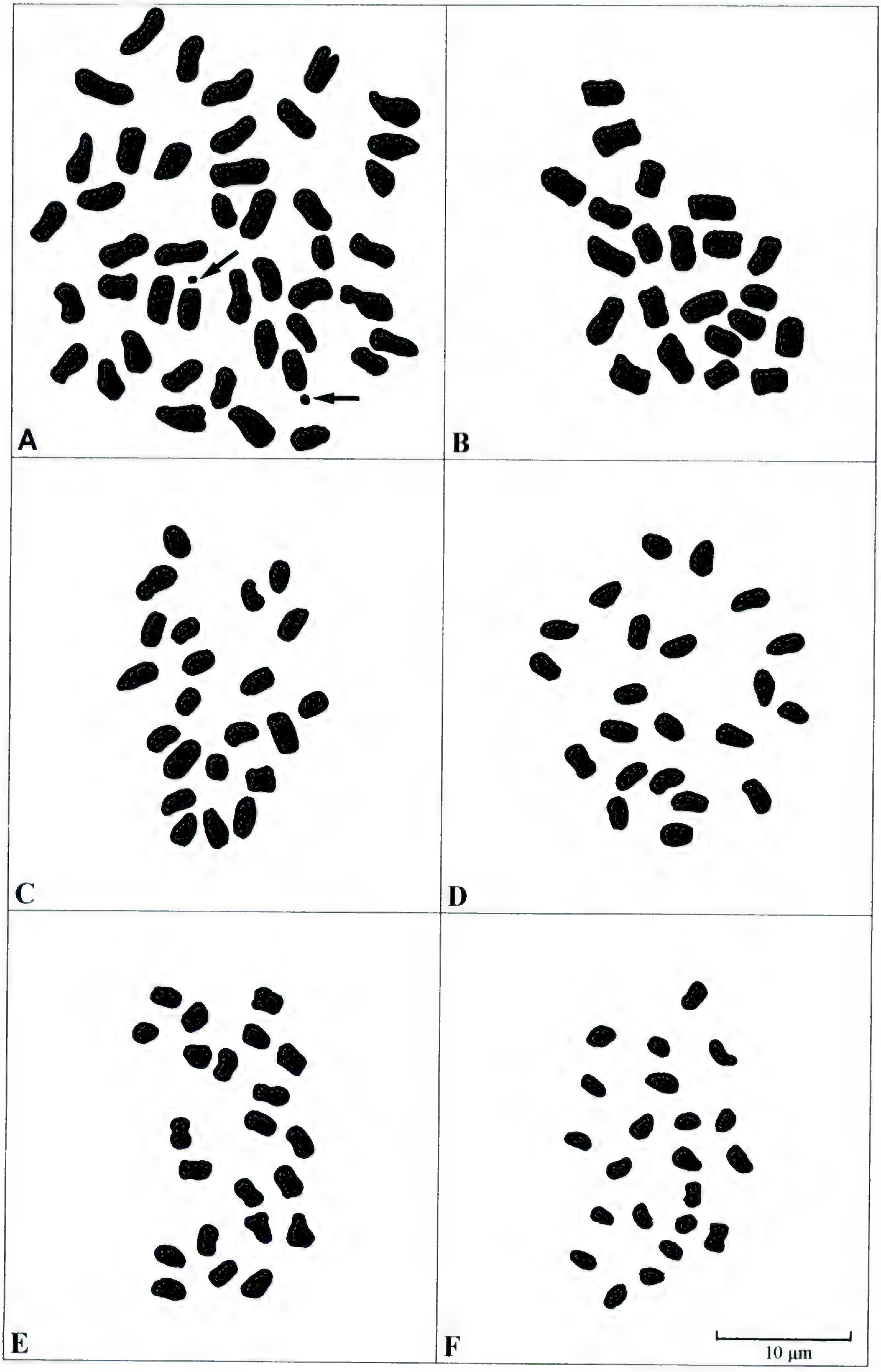
The relative 2C DNA values for over 30 taxa of *Caralluma*, *Ceropegia*, *Duvalia*, *Orbea*, *Stapelianthus*, and *Tridentea* have been cytophotometrically determined in our lab and compared to the average karyotype lengths (cf. Huesmann, 1993). All our studies showed a good correlation between the two parameters of DNA content and chromosome size. For example, in the *Caralluma* complex we found for *C. umbellata* 0.82 pg/20.4 μm, *C. diffusa* 1.18 pg/27.6 μm, *C. europaea* 1.38 pg/33.7 μm, *C. sarkariae* 1.43 pg/18.9 μm, *C. turneri* 1.50 pg/24.3 μm, and for *C. edulis* 1.83 pg/27.8 μm (Huesmann, 1993). The differences between some of these values are considerable, supporting, for example, the separation of *Caralluma* s.l. into a handful of smaller genera as will be proposed by Meve and Liede (unpublished) based on DNA sequence data. Determinations of 4C DNA values, as investigated by Pattnaik et al. (1997) for six stapeliad species, were not studied by us. Whereas the karyotype lengths measured by these authors are in the range of our results, the DNA values estimated are by far higher than ours. The reasons for these deviations are unclear.

GEOGRAPHICAL DISTRIBUTION OF DIFFERENT BASIC CHROMOSOME NUMBERS

We have contributed most to the karyological knowledge in African Asclepiadoideae since the emphasis of our ongoing study was on the Ceropegieae. In contrast, in other regions of the world there are still many gaps in the data. Despite this geographically biased data set of basic chromosome numbers, some summary can be made to show their diversity in the different regions of the world (Table 2).

The Asclepiadoideae are worldwide in distribution, whereas the primitive Periplocoideae and Secamonoideae are restricted to the Old World. Notably, all taxa investigated of the latter two subfamilies, and also the basal Fockeeae of the Asclepiadoideae, have $x = 11$, large chromosomes, and they all come from Africa (only one species from Arabia).

In Europe, Madagascar, and Australia only the basic number $x = 11$ is known. In the best studied area, Africa, just a single genus, the southern African *Microloma*, has the deviating basic chromosome number of $x = 10$ (Albers et al., 1993). The African *Pentarrhinum insipidum* is variable in its basic chromosome number ($x = 9$, 11, and 12), although the $2n = 18$ reported from Burundi (Renard et al., 1983) remains mysterious because otherwise only increased numbers have been found for this species. Additionally, $x = 13$ has been counted once by Bramwell et al. (1972) for *Calotropis procera* from the Cape Verde Islands, whereas Borgen (1975) confirmed the counts of $x = 11$ found also by other authors (e.g., Bhattacharya et al., 1971). We also counted $x = 11$ for the same taxon and even from Cape Verde. In all cases where counts of $2n = 24$ have been found, 2 B-chromosomes are most likely responsible, due to their inconsistency of occurrence; variation between $2n = 22$ and $2n = 24$ occurs among populations (*Pentarrhinum insipidum*) and also within populations (e.g., *Brachystelma mortonii*, Appendix 1) corroborating this concept (cf. Rees, 1974). One or two B-chromosomes have been found to occur commonly in *Hoya* (Nakamura, 1993). In Asia the percentage of species with deviating numbers ($x = 12$ have been reported for, e.g., *Heterostemma tanjorense* Wight & Arn. [Navaneetham, 1981] or *Tylophora asthmatica* Wight & Arn. [Sreedevi & Namboodiri, 1977]), is slightly higher than in the rest of the Old World, but strikingly different from the situation found in


A
B
C
D
E
F
10 µm

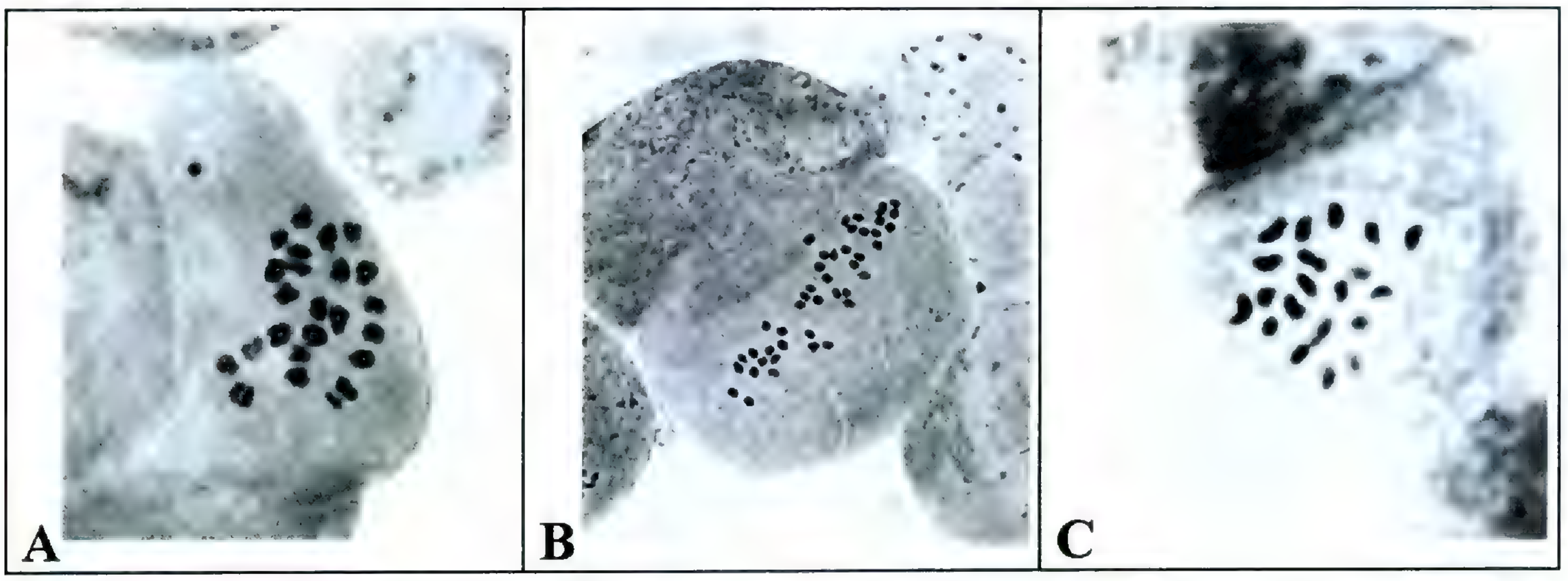


Figure 3. Mitotic metaphase chromosomes of Asclepiadoideae in the light microscope. —A. *Ceropegia cumingiana*, $2n = 22$ (*Liede 3250*). —B. *Caralluma adscendens* var. *gracilis*, $2n = 44$ (*Sarkaria 32-90*). —C. *Microloma incanum*, $2n = 20$ (*Albers & Meve 88*). (A: ca. 1350×; B: ca. 500×; C: ca. 2400×; for vouchers see Appendix 1.)

the New World (Table 2). Here, one fifth of the species in North America (incl. Mexico) and more than one third in southern Central and South America have derived, predominantly decreased numbers (e.g., *Grisebachiella, Orthosia, Philibertia*; Appendix 1). It seems that chromosomal evolution with respect to the basic numbers has begun a new stage in the New World.

Discussion

Different basic chromosome numbers often discriminate a taxon into separate taxonomical subunits such as families, tribes, or genera (various examples are given in Stace, 1989). Many studies have demonstrated the taxonomic value of basic chromosome numbers even at infrageneric level (e.g., *Pelargonium*: Albers et al., 1992; *Moraea*: Goldblatt, 1971, 1986). As van der Laan and Arends (1985) indicated, the basic number $x = 11$ is the most frequent number in Apocynaceae s. str. (Apocynoideae & Rauvolfioideae). Approximately 60% of the species they studied have this number, but 40% deviate (van der Laan & Arends, 1985). The numbers $x = 10$, 9, 8, 7, and 6 have evolved by descending aneuploidy, whereas $x = 12$ is an increasing number (van der Laan & Arends, 1985). One fifth of all studied species contain the deviating number $x = 9$. This high proportion might be inflated by the relatively high number of karyologically studied species in the genera *Pachypodium* and *Strophanthus*. At present karyological information is only available for 241 species (14%) of Apocynaceae s. str. from 73 genera (40%) (van der Laan & Arends, 1985; Albers, unpublished data). Although this rate seems rather poor, it represents quite a typical situation for a family with predominantly woody species (e.g., Ehrendorfer, 1982).

The only basic chromosome number in the Periplocoideae and Secamonoideae is $x = 11$, which is also by far the most common situation in the Asclepiadoideae. Comparing these basic chromosome numbers with those of the Apocynaceae s. str. there is one striking difference: deviating basic chromosome numbers occur much less frequently in Asclepiadoideae with only about 4% compared with 40% in the other two subfamilies Apocynoideae and Rauvolfioideae.

The relatively frequent occurrence of different basic numbers in various tribes and subtribes of both Apocynoideae and Rauvolfioideae points to parallel genome evolution (van der Laan & Arends, 1985). The main developments have taken place repeatedly but independently.

In Apocynaceae s. str. polyploidy is only known to occur in nine genera (van der Laan & Arends, 1985). In total, 13% of the Apocynaceae s. str. are

←

Figure 2. Squash preparations of mitotic metaphase plates (camera lucida drawings). —A. Periplocoideae: *Raphionacme hirsuta* ($2n = 44$, two satellites marked by arrows, *Venter 9110*). —B. Secamonoideae: *Secamone punctulata* ($2n = 22$, *Hemp s.n.*). —C. Asclepiadoideae, Fockeeae: *Cibirhiza dhofarensis* ($2n = 22$, *Butler s.n.*). —D. Asclepiadoideae, Marsdenieae: *Telosma accedens* ($2n = 22$, *Schneidt 96-45*). —E. Asclepiadoideae, Ceropegieae: *Angolluma denboefii* ($2n = 22$, *Masinde 889*). —F. Asclepiadoideae, Asclepiadinae: *Sarcostemma viminale* subsp. *odontolepis* ($2n = 22$, *Meve & Liede 3354*). (Bar = 10 μm; for vouchers see Appendix 1.)

Table 2. Geographical distribution of basic chromosome numbers in Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae).

Region	No. of taxa[1] studied	Basic chromosome numbers of karyologically known taxa in percentages					
		x = 9	x = 10	x = 11	x = 12	x = 13	x = 14
Old World							
Africa & Arabia	496	0.2	0.8	98.6	0.2	0.2	—
Europe	9	—	—	100	—	—	—
Asia	83	—	—	95.2	4.8	—	—
Australia	9	—	—	100	—	—	—
Madagascar	63	—	—	100	—	—	—
New World							
North America incl. Mexico	37	—	18.9	81.1	—	—	—
S Central & South America	30	10	23.3	63.4	—	—	3.3

[1] Each taxon is scored in every region of its occurrence.

polyploids, which is comparatively little (cf. Morton, 1966). All basic chromosome numbers are involved in different polyploid series. The highest number ever counted are the ca. 180 chromosomes of *Alyxia* (van der Laan & Arends, 1985). The situation in Asclepiadoideae is very similar, but the degree of polyploids is even lower, with only around 6%. Only in the Ceropegieae, which are dominated by the stem-succulent stapeliads, does polyploidy increase to about 10%, supporting the old hypothesis of the better ability of polyploids at colonizing new areas and being more hardy in less favorable habitats (e.g., Stebbins, 1985). However, compared to the 27.9% polyploid taxa in Cactaceae (Pinkava et al., 1985), it cannot be argued that polyploidy really played an important role in stapeliad evolutionary history. This might be true only on a more regional scale. The stapeliads had their evolutionary center in northeastern Africa (Meve, 1997a), but they formed a secondary center in southern Africa, where the majority of stapeliad polyploids are distributed (Albers, 1983; Albers & Meve, 1991, unpublished data). Here, rapid speciation took place accompanied not by derivation of chromosome number but instead by an increase in polyploidization events. This means that speciation here has taken place only at the level of chromosome and not of the karyotype (genome evolution). In contrast, the Apocynaceae s. str. have been more successful with derived numbers (karyotype evolution).

Tetraploidy is by far the most frequent polyploid condition in both Apocynaceae s. str. and Asclepiadoideae. In general, the tetraploid condition is considered to be the most successful in higher plants (DeWet, 1980). Even Cactaceae do not depart from this, since infraspecific polyploidy, mainly based on autopolyploidy, is rare in the entire family (cf. Pinkava et al., 1985).

Van der Laan and Arends (1985) observed that most chromosomes of Apocynaceae s. str. (Apocynoideae and Rauvolfioideae) species fall in the range of 1 to 2 μm in length. Considerably longer chromosomes are found only in the tribes Alstonieae and Plumerieae (*Vallesia* and *Allamanda*, up to 3 μm long) and Tabernaemontaneae (*Tabernaemontana* and *Voacanga*, up to 3.5. μm long) of the Rauvolfioideae (data extracted from van der Laan & Arends, 1985; tribal disposition sensu Endress & Bruyns, 2000), and could indicate a similar trend in Asclepiadoideae, with larger chromosomes being predominantly confined to basal taxonomic units. On the average, however, there is no noticeable difference in chromosome length between the two subfamilies of Apocynaceae s. str. In both, average chromosome length is around 1.5 μm. The chromosomes of the generally woody Apocynaceae s. str. can be considered as small and are in the same range as those of the Periplocoideae, Secamonoideae, and some tribes of the Asclepiadoideae. Pattnaik et al. (1997), Raynaud (1991), and Nakamura (1991) published measurements of similar sizes for some stapeliads. However, the analysis of Pattnaik et al. (1997: Table 1, p. 89) revealed larger rather than smaller chromosomes in tetraploids as compared with diploids. For example, Pattnaik gave the karyotype length for *Orbea paradoxa* as 61.54 μm, whereas we measured 42.91 μm (Albers & Meve, unpublished). This surprising difference, which is even higher when comparing tetraploids with diploids of *Orbea variegata*, remains unexplained.

Comparing chromosome size in the Periplocoideae, Secamonoideae, and Asclepiadoideae, on av-

erage the size of the karyotype diminishes stepwise starting with rather large chromosomes in the basal Periplocoideae and ending with the smallest karyotype length in the presumedly most advanced tribe of the Asclepidoideae, the Asclepiadeae. In the asclepiads reduction of the genome size ($x = 10$, $x = 9$) is restricted to the tribe Asclepiadeae, which is also the only group characterized by having pendent pollinia. All other taxa (Secamoneae, Fockeeae, Marsdenieae, and Ceropegieae) have the conserved $x = 11$, and are characterized by having erect pollinia. The erect position of pollinia has been shown to represent the primitive state in Asclepiadoideae by Kunze's (1993) careful morphological analysis. Within the tribe Asclepiadeae, subtribe Gonolobineae can be regarded as the karyologically most primitive, since its chromosomes are on the average slightly more than 1 μm long—the largest in Asclepiadeae, and approaching the length of those in the Ceropegieae. Gonolobinae also do not have numbers deviating from $x = 11$. This is an interesting result for the systematics of the subfamily Asclepiadoideae, since erect as well as more or less pendent, but mostly horizontally arranged pollinia have been reported for the Gonolobinae (cf. Kunze, 1995). Thus, both pollinarium morphology as well as karyological data provide evidence for a basal position of the Gonolobinae within the Asclepiadeae, and the results of the large cp DNA study by Potgieter and Albert (2001 this volume) do not refute this. Because they are restricted to the most advanced groups of the Asclepiadoideae, the subtribes Astephaninae, Metastelminae, and Oxypetalinae of Asclepiadeae, we regard reductions in the basic chromosome number as a relatively recent step in genome evolution within Asclepiadeae, which has taken place in parallel in the Old World (Astephaninae) and the New World (Metastelminae and Oxypetalinae).

Variation in the karyotype length is not restricted to the tribal level; it is also widespread at the generic level. In large genera, e.g., *Ceropegia* and *Brachystelma*, karyotype lengths can vary considerably from species to species, without compromising the generic affiliation of species with deviating chromosome size, such as *Brachystelma* (*Macropetalum*) *burchellii* (cf. Meve & Liede, 2001a). In small genera or groups of species considerable differences in chromosome size between the species might, however, question congeneric treatments. For example, the inclusion of *Larryleachia* (1.40 μm/chromosome length on average) in *Lavrania* (0.91 μm), as proposed by Bruyns (1999), is rejected by Meve and Liede (2001b). And in *Caralluma* s.l. karyotype length analysis will support new taxonomic solutions for this heterogeneous assemblage of species. In particular, *C. edulis* (ca. 1.5 μm) and *C. sinaica* (ca. 1.3 μm, Albers & Meve, unpublished data), which came out as a separate group in molecular studies (Meve & Liede, unpublished data), are additionally characterized by their large chromosomes. As to the direction in chromosome size evolution (from large to small) in the Asclepiadoideae as postulated above, the results we have for *Neoschumannia* support our ideas. *Neoschumannia*, which has the largest chromosomes in subfamily Asclepiadoideae (1.72 μm in *N. kamerunensis*) but a complex corona that is 3-seriate, has been regarded as a primitive member of the Ceropegieae (Meve, 1995b, 1997b).

In Marsdenieae we again have support for the hypothesis that basal taxa tend to have large chromosomes, since Omlor (1998) regarded the genera *Marsdenia* and *Telosma* (those with large chromosomes) as basal in the tribe. In contrast, the highly diverse and specialized Asian/Australian genus *Hoya*, where many of the up to 300 species (Forster et al., 1998) show derived features as such as herbaceous epiphytic growth, succulent leaves, clear latex, and a germination crest along the inner edge of the pollinium, possesses rather small chromosomes (0.91 μm on average for the genus, Fig. 1; for sizes of *Hoya* chromosomes see also Nakamura, 1992, 1993).

Considering the distribution patterns of basic chromosome numbers and of the different karyotype sizes in Asclepiadoideae, our data suggest Africa as the common area where (karyotype) evolution began. This conclusion coincides with that of van der Laan and Arends (1985) for Apocynoideae and Rauvolfioideae, where most of the genera with derived basic chromosome numbers occur outside the African continent (cf. *Catharanthus* and *Apocynum* ($x = 8$) in Madagascar, the Middle East, and North America; *Plumeria* and *Allamanda* ($x = 9$) in the Americas; *Odontadenia* and *Beaumontia* ($x = 12$) in America and Asia). Of the ca. 40 genera of Apocynaceae s. str. occurring in Africa, 35 have been karyologically investigated. Of these, only 3 are not characterized by $x = 11$, again supporting the idea of an African origin of the Apocynaceae s.l.

Literature Cited

Albers, F. 1974. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports XLIV. Taxon 23: 373–380.

———. 1975. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports XLIX. Taxon 24: 501–516.

———. 1976. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports LI. Taxon 25: 155–164.
———. 1977. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports LVIII. Taxon 26: 557–558.
———. 1981. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports LXX. Taxon 30: 68–80.
———. 1983. Cytotaxonomic studies in African Asclepiadaceae. Bothalia 14: 795–798.
——— & M. Austmann. 1987. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports. Taxon 36: 494–496.
——— & W. Delfs. 1983. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports LXXXI. Taxon 32: 667–668.
——— & U. Meve. 1991. Mixoploidy and cytotypes: a study of possible vegetative species differentiation in stapeliads (Asclepiadaceae). Bothalia 21: 67–72.
———, M. Austmann & U. Meve. 1988. Asclepiadaceae. *In* C. Stace (editor), Chromosome number report. IOPB Newslett. 11: 10–12.
———, ——— & ———. 1990. Asclepiadaceae. *In* C. Stace (editor), IOPB Chromosome data 2. IOPB Newslett. 15: 11–14.
———, M. Gibby & M. Austmann. 1992. A reappraisal of *Pelargonium* sect. *Ligularia* (Geraniaceae). Pl. Syst. Evol. 179: 257–276.
———, S. Liede & U. Meve. 1993. Deviating chromosome numbers in Asclepiadaceae. Nordic J. Bot. 13: 37–39.
Bennett, M. D. & J. B. Smith. 1976. Nuclear DNA amounts in Angiosperms. Philos. Trans., Ser. B 274: 227–274.
Bhattacharya, S. S., M. M. Khalifa & I. I. Chaudhari. 1971. Asclepiadaceae. *In* A. Löve (editor), IOPB Chromosome number reports XXXII. Taxon 20: 349–356.
Borgen, L. 1975. Chromosome numbers of Macaronesian flowering plants. Norweg. J. Bot. 22: 71–76.
Bramwell, D., C. J. Humphries, B. G. Murray & S. J. Owens. 1972. Chromosome studies in the flora of Macaronesia. Bot. Not. 125: 139–152.
Brown, R. 1810. On the Asclepiadeae, a natural order of plants separated from the Apocineae of Jussieu. [Preprint of Mem. Wern. Nat. Hist. Soc. 1: 12–78. 1811.]
Bruyns, P. V. 1999. The status of *Larryleachia* Plowes and additional synonymy for *Lavrania* Plowes. S. African J. Bot. 65: 302–305.
——— & P. I. Forster. 1991. Recircumscription of the Stapelieae (Asclepiadaceae). Taxon 40: 381–391.
Darlington, C. D. & A. P. Wylie (Editors). 1955. Chromosome Atlas of Flowering Plants. Allen & Unwin, London.
DeWet, J. M. J. 1980. Origin of polyploids. Pp. 3–15 *in* W. H. Lewis (editor), Polyploidy: Biological Relevance. Plenum, New York.
Ehrendorfer, F. 1982. Speciation patterns in woody Angiosperms of tropical origin. *In:* C. Barigozzi (editor), Mechanisms of Speciation, Progress in Clinical and Biological Research 96: 479–509. Liss, New York.
Endress, M. E. & P. V. Bruyns. 2000. A revised classification of the Apocynaceae s.l. Bot. Rev. (Lancaster) 66: 1–56.
Fedorov, A. A. 1974. Chromosome Numbers of Flowering Plants. Otto Koeltz Science Publishers, Koenigstein.
Forster, P. I., D. J. Liddle & I. M. Liddle. 1998. Diversity in the genus *Hoya* (Asclepiadaceae–Marsdenieae). ALOE 35(2): 44–48.
Goldblatt, P. 1971. Cytological and morphological studies in southern African Iridaceae. J. S. African Bot. 37: 317–460.
——— (Editor). 1981. Index to plant chromosome numbers 1975–1978. Monogr. Syst. Bot. Missouri Bot. Gard. 5: 1–553.
——— (Editor). 1984. Index to plant chromosome numbers 1979–1981. Monogr. Syst. Bot. Missouri Bot. Gard. 8: 1–427.
——— (Editor). 1985. Index to plant chromosome numbers 1982–1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13: 1–224.
———. 1986. The Moraeas of southern Africa. Ann. Kirstenbosch Bot. Gard. 14.
——— (Editor). 1988. Index to plant chromosome numbers 1984–1985. Monogr. Syst. Bot. Missouri Bot. Gard. 23: 1–264.
——— & D. E. Johnson (Editors). 1990. Index to plant chromosome numbers 1986–1987. Monogr. Syst. Bot. Missouri Bot. Gard. 30: 1–243.
——— & ——— (Editors). 1991. Index to plant chromosome numbers 1988–1989. Monogr. Syst. Bot. Missouri Bot. Gard. 40: 1–238.
——— & ——— (Editors). 1994. Index to plant chromosome numbers 1990–1991. Monogr. Syst. Bot. Missouri Bot. Gard. 51: 1–267.
——— & ——— (Editors). 1996. Index to plant chromosome numbers 1992–1993. Monogr. Syst. Bot. Missouri Bot. Gard. 58: 1–276.
——— & ——— (Editors). 1998. Index to plant chromosome numbers 1994–1996. Monogr. Syst. Bot. Missouri Bot. Gard. 69: 1–208.
Greilhuber, J. 1986. Severely distorted Feulgen–DNA amounts in *Pinus* (Coniferophytina) after nonadditive fixation as a result of meristematic self-tanning with vacuole contents. Canad. J. Genet. Cytol. 28: 409–415.
Huesmann, R. 1993. Cytologische und karyologische Untersuchungen innerhalb der Tribus Stapelieae. Diploma thesis, University of Münster, Münster. Unpublished.
Klackenberg, J. In press. Notes on Secamonoideae (Apocynaceae) in Africa. Adansonia 23(2).
Kunze, H. 1993. Evolution of the translator in Periplocaceae and Asclepiadaceae. Pl. Syst. Evol. 185: 90–122.
———. 1995. Floral morphology of some Gonolobeae (Asclepiadaceae). Bot. Jahrb. Syst. 117: 211–218.
Laan, F. M. van der & J. C. Arends. 1985. Cytotaxonomy of the Apocynaceae. Genetica 68: 3–35.
Lewis, W. H. (Editor) 1980. Polyploidy. Biological Relevance. Plenum Press, New York.
Liede, S. 1997. Subtribes and genera of the tribe Asclepiadeae (Apocynaceae, Asclepiadoideae)—A synopsis. Taxon 46: 233–247.
———. 2001. Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) reconsidered: New evidence based on cpDNA spacers. Ann. Missouri Bot. Gard. 88: 657–668.
——— & U. Meve. 1993. Towards an understanding of the *Sarcostemma viminale* (Asclepiadaceae) complex. Bot. J. Linn. Soc. 112: 1–15.
——— & ———. 1995. The genus *Sarcostemma* R.Br. (Asclepiadaceae) in Madagascar. Bot. J. Linn. Soc. 118: 37–51.
Meve, U. 1995a. Cytological and morphological differentiation in *Caralluma burchardii* N.E.Br. (Asclepiadaceae). Nordic J. Bot. 15: 459–467.
———. 1995b. *Neoschumannia* (including *Sucynnertonia*), a primitive genus of the Asclepiadaceae–Stapelieae. Pl. Syst. Evol. 197: 233–242.

———. 1997a. The Genus *Duvalia* (Asclepiadaceae)—Stem-Succulents between the Cape and Arabia. Springer, Vienna.

———. 1997b. *Neoschumannia kamerunensis* Schltr. (Asclepiadaceae) rediscovered in Cameroon after nearly a century. Bot. Jahrb. Syst. 119: 427–435.

———. 1999. *Tylophora anomala* (Asclepiadaceae)—A cytologically anomalous species. Syst. Geogr. Pl. 68: 255–263.

——— & S. Liede. 1996. *Sarcostemma* R.Br. (Asclepiadaceae) in East Africa and Arabia. Bot. J. Linn. Soc. 120: 21–38.

——— & ———. 2001a. Inclusion of *Tenaris* and *Macropetalum* in *Brachystelma* (Apocynaceae–Asclepiadoideae–Ceropegieae) inferred from non-coding nuclear and chloroplast DNA sequences. Pl. Syst. Evol. 228: 89–105.

——— & ———. 2001b. Reconsideration of the status of *Lavrania*, *Larryleachia* and *Notechidnopsis* (Asclepiadoideae-Ceropegieae). S. African J. Bot. 67: 161–168.

Moore, R. J. (Editor). 1973. Index to plant chromosome numbers 1967–1971. Regnum Veg. 90: 1–539.

——— (Editor). 1974. Index to plant chromosome numbers for 1972. Regnum Veg. 91: 1–108.

——— (Editor). 1977. Index to plant chromosome numbers for 1973/74. Regnum Veg. 96: 1–257.

Morton, J. K. 1966. The role of polyploidy in the evolution of a tropical flora. Pp. 73–76 *in* C. D. Darlington & K. R. Lewis (editors), Chromosomes Today, Vol. 1. Oliver & Boyd, Edinburgh.

Nakamura, T. 1991. A study of geographical differention and chromosomes of succulent plants in the family Asclepiadaceae. Kromosomo 2.61: 2068–2077.

———. 1992. Cytological studies on 13 species of genus *Hoya* (Asclepiadaceae) in Malay Peninsula. Chromosome Inform. Serv. 53: 16–19.

———. 1993. Cytological studies on 6 species of the genus *Hoya* (Asclepiadaceae) in Viti Levu Island, Fiji. Chromosome Inform. Serv. 55: 25–27.

Navaneetham, N. 1981. Chromosome numbers. *In* A. Löve (editor), IOPB chromosome number reports LXXII. Taxon 30: 694–708.

Olmstead, R. G., B. Bremer, K. M. Scott & J. D. Palmer. 1993. A parsimony analysis of the Asteridae sensu lato based on *rbc*L sequences. Ann. Missouri Bot. Gard. 80: 700–722.

Omlor, R. 1998. Generische Revision der Marsdenieae (Asclepiadaceae). Shaker, Aachen.

Ornduff, R. (Editor). 1967. Index to plant chromosome numbers for 1965. Regnum Veg. 50: 1–128.

Ornstein, L. 1952. The distributional error in microspectrophotometry. Lab. Invest. 1: 250–265.

Patau, K. 1952. Absorption microphotometry of irregulary shaped objects. Chromosoma 5: 341–362.

Pattnaik, M., A. B. Das & P. Das. 1997. Karyotype analysis and 4C DNA variability in some succulent members of the tribe Stapelieae. Cytobios 91: 87–95.

Pinkava, D. J., M. A. Baker, D. Parfitt, M. W. Mohlenbrock & R. D. Worthington. 1985. Chromosome numbers in some cacti of western North America—V. Syst. Bot. 10: 471–483.

Potgieter, K. & V. A. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.

Raynaud, C. 1991. Morphologie, caryologie et biogéographie de deux endémiques marocaines: *Caralluma hesperidum* Maire et *C. joannis* Maire (Asclepiadaceae). Bot. Chron. 10: 713–722.

Rees, H. 1974. B chromosomes. Sci. Progr. (Oxford) 61: 535–554.

Reese, G. 1971. Untersuchungen über die Chromosomenzahlen der Stapelieae II. Portugaliae Acta Biol., Sér. A, 12(1–2): 1–23.

——— & H. Kressel. 1967. Untersuchungen über die Chromosomenzahlen der Stapelieae. Portugaliae Acta Biol., Sér. A, 10(1–2): 33–54.

Renard, R., J. Lambinon, M. Reekmans, P. v.d.Veken & M. Govaert. 1983. Nombres chromosomiques de quelques Angiospermes du Rwanda, du Burundi et du Kenya. Bull. Jard. Bot. Belg. 53: 343–371.

Schlechter, R. 1905. Periplocaceae. Pp. 351–353 *In* T. C. Fries & R. Fries (editors), Nachträge zur Flora der Deutschen Schutzgebiete in der Südsee. Borntraeger, Leipzig.

Sennblad, B. & B. Bremer. 1996. The familial and subfamilial relationships of Apocynaceae and Asclepiadaceae evaluated with *rbc*L data. Pl. Syst. Evol. 202: 153–175.

Snow, R. 1963. Alkoholic acid-carmine as a stain for chromosomes in squash preparations. Stain Technol. 38: 9–13.

Sreedevi, P. & A. N. Namboodiri. 1977. Chromosome numbers. *In* A. Löve (editor), IOPB chromosome number reports LVI. Taxon 26: 257–274.

Stace, C. A. 1989. Plant Taxonomy and Biosystematics, 2nd ed. Arnold, London.

Stebbins, G. L. 1971. Chromosomal Evolution in Higher Plants. Arnold, London.

———. 1985. Polyploidy, hybridization, and the invasion of new habitats. Ann. Missouri Bot. Gard. 72: 824–832.

Tjio, J. H. & A. Levan. 1950. The use of oxyquinoline in chromosome analysis. Anales Estac. Exp. Aula Dei 2: 21–64.

Venter, H. J. T. 1997. A revision of *Periploca* (Periplocaceae). S. African J. Bot. 63: 123–128.

——— & R. L. Verhoeven. 1997. A tribal classification of the Periplocoideae (Apocynaceae). Taxon 46: 705–720.

——— & ———. 2001. Diversity and relationships within the Periplocoideae (Apocynaceae). Ann Missouri Bot. Gard. 88: 550–568.

Appendix 1. New chromosome numbers in Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae). The table is differentiated in the two categories: (1) New and first chromosome counts for taxon (unmarked); and (2) New counts confirming previous chromosome numbers (marked by #); additionally, taxa marked by an asterix (*) served as vouchers for chromosome size analysis, but their chromosome numbers were published previously.

Taxon	2*n*	Locality	Voucher
PERIPLOCOIDEAE			
***Camptocarpus* Decne.**			
C. crassifolius Decne.	22	MADAGASCAR: s. loc.	*Petignat s.n.* [ULM]
***Cryptolepis* R. Br.**			
C. decidua (Planch. ex Hook. f. & Benth.) N. E. Br.	22	NAMIBIA: Warmbad	*Verhoeven 203*
C. oblongifolia (Meisn.) Schltr.	22	R.S.A.: Eastern Cape Prov., Vredefort	*du Preez s.n.*
C. sanguinolenta (Lindl.) Schltr.	22	NIGERIA: s. loc.	*ex hort.* BG MSUN [MSUN]
***Cryptostegia* R. Br.**			
C. madagascariensis Bojer ex Decne.	22	s. loc.	*ex hort* MSUN
***Ectadium* E. Mey.**			
E. virgatum E. Mey. var. *virgatum*	22	NAMIBIA: 16 km O Lüderitz	*Albers 2413* [MSUN]
***Mondia* Skeels**			
M. whitei (Hook. f.) Skeels	22	TANZANIA: S slope Mt. Kilimanjaro, Msaranga Valley	*Liede & Meve 3351* [UBT]
***Periploca* L.**			
P. aphylla Decne.	22	ISRAEL: Daragoth (Dead Sea Area), near road to Ma'aleh	*Arishai s.n.*
P. visciformis K. Schum.	44	YEMEN: Jabal Haraz, ca. 25 km S Manákhah	*Mangelsdorff Y15* [in hort UBT]
***Petopentia* Bullock**			
P. natalensis (Schltr.) Bullock	22	*ex hort.* Kiel	*ex hort. Kiel*
***Raphionacme* Harv.**			
R. brownii Scott-Elliott	22	IVORY COAST (STATE): Korhogo, Granitinselberg (IB2)	*Porembski & Biedinger 1488* [MSUN]
R. flanaganii Schltr.	22	R.S.A.: Kwazulu-Natal, Pietermaritzburg, S Ashburn Stn.	*Albers & Balkwill in K 1577* [MSUN]
R. globosa K. Schum.	22	TANZANIA: Ruvuma Prov.	*Specks 286* [MSUN]
R. hirsuta (E. Mey.) R. A. Dyer	44	R.S.A.: Northern Prov., Sterkfontein caves	*Venter 9110*
R. lanceolata Schinz	22	IVORY COAST (STATE): s. loc.	*Porembski 271* [MSUN]
R. madiensis S. Moore	22	TANZANIA: Ruvuma Prov., Namchwea Hill	*E. & M. Specks 494* [MSUN]
R. palustris Venter & R. L. Verh.	44	R.S.A: s. loc.	*Stork 24* ex hort MSUN
R. splendens Schltr.	22	TANZANIA: s. loc.	*Specks 692* [MSUN]
***Schlechterella* K. Schum.**			
S. abyssinica (Chiov.) Venter & R. L. Verh.	22	KENYA: Forole	*Newton 4555* [EA]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Stomatostemma* N. E. Br.**			
S. monteiroae N. E. Br.	22	s. loc.	*s.n. sub IPPS 1427* [MSUN]
***Tacazzea* Decne.**			
T. apiculata Oliv.	22	R.S.A.: Northern Prov.	*Venter 9188* [K]
SECAMONOIDEAE			
***Secamone* R. Br.**			
S. alpinii Schult.	22	R.S.A.: Western Cape Prov., Leipoldt's Grave	*Liede 2548*
S. filiformis (L. f.) J. H. Ross	22	R.S.A.: Northern Prov., Venda	*Liede 632* [K, MSUN]
S. parviflora (Oliv.) Bullock	22	KENYA: Kwale, Shimoni	*Liede & Newton 3196* [E, MSUN, ULM]
S. parviflora (Oliv.) Bullock	44	KENYA: Kilifi, GEDE National Monument	*Liede & Newton 3198*
S. punctulata Decne.	22	TANZANIA: Moshi, Lake Chala	*A. & C. Hemp s.n.* [UBT]
ASCLEPIADOIDEAE			
Tribe: FOCKEEAE			
***Cibirhiza* Bruyns**			
* *C. albersiana* Kunze, Meve & Liede	22	TANZANIA: Dodoma Prov., 5 km S Mpwapwa	*Specks 248*
C. dhofarensis Bruyns	22	OMAN: Dhofar, 25 km W Salalah	*Butler s.n.* [UBT]
***Fockea* Endl.**			
F. angustifolia K. Schum.	22	ZIMBABWE: Hwange, Deka Matetsi	*Albers & Meve 524*
F. capensis Endl.	22	R.S.A.: Western Cape Prov., s. loc.	*ex hort. Kiel* [MSUN]
F. edulis (Thunb.) K. Schum.	22	R.S.A.: s. loc.	*s. coll.* [MSUN]
F. sinuata (E. Mey.) E. A. Bruce	22	R.S.A.: Eastern Cape Prov., Willowmore	*Bruyns 5075*
Tribe: MARSDENIEAE			
***Cionura* Griseb.**			
C. erecta (L.) Griseb.	22	TURKEY: Side, Manaugat	*Heyne 120* [UBT]
***Dischidia* R. Br.**			
D. bengalensis Colebr.	22	s. loc.	*Cumming s.n.* [MSUN]
D. hirsuta L.	22	PHILIPPINES: Quezon Natl. Park	*S. Schneidt s.n.* [ULM]
D. (Dischidiopsis) luzonica Schltr.	22	PHILIPPINES: Mt. Makiling	*Liede 3262*
D. nummularia Blume	22	PHILIPPINES: Quezon Natl. Park, from Pagbilao to Atimonan	*Schneidt & Liede 96-18* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Dregea* E. Mey.**			
D. rubicunda K. Schum.	22	KENYA: GEDE National Monument, Kilifi	*Liede & Newton 3199* [ULM]
D. schimperi (Decne.) Bullock	22	ZIMBABWE: Thomson Junction between Victoria Falls and Hwange	*Albers, Liede & Meve 520*
***Gymnema* R. Br.**			
# *G. sylvestre* R. Br.	22	CAMEROON: 20 km E Mokolo	*Meve 919* [B, UBT]
***Hoya* R. Br.**			
H. australis R. Br. ex Traill	22	s. loc.	*van Donkelaar s.n.* [in hort UBT]
H. camphorifolia Warburg	22	PHILIPPINES: Quezon, Quezon National Park	*Schneidt 96-20* [UBT]
H. heuschkeliana Kloppenburg	22	PHILIPPINES: Luzon, Lake Bulusan	*Schneidt 96-96* [UBT, ULM]
# *H. longifolia* Wall.	22	*ex hort.* BG Münster	*Helmcker s.n.* [in hort MSUN]
H. meliflua Merr.	22	PHILIPPINES: Luzon, Mt. Makiling	*Liede 3274* [MSUN, ULM]
***Marsdenia* R. Br.**			
M. linearis Decne.	22	CUBA: s. loc.	*s. coll.*
M. megalantha Goyder & Morillo	22	BRAZIL: Bahia, Mun. Iramaia, 12 km W Martacas towards Iramaia	*Taylor et al. 1557* [K, MO, MSUN]
M. rostrata R. Br.	22	AUSTRALIA: NSW, 2 km S Kioloa	Meve & Hellbrügge 791 [MSUN]
***Telosma* Coville**			
T. cordata Merr.	22	PHILIPPINES: Luzon, Alaminos, Hundred Islands Natl. Park	*Schneidt & Liede 96-105* [AUH]
T. accedens Blume	22	PHILIPPINES: Laguna Prov. Mt Makiling	*Schneidt 96-45* [UBT]
Tribe: CEROPEGIEAE			
***Angolluma* Munster (= *Pachycymbium* Leach *p.p.*, = *Stultitia* Phillips)**			
# *A. abayensis* (M. G. Gilbert) Plowes cf.	22	ETHIOPIA: Lake Abaye	*Frank 64* [MSUN]
A. commutata (A. Berger) Plowes	22	SAUDI ARABIA: s. loc.	*Collenette 8255*
# *A. decaisneana* (Lem.) L. E. Newton	22	ALGERIA: Tasille (Sahara)	*Plowes 3971*
A. denboefii (Lavranos) Plowes	22	TANZANIA: Himo	*Masinde 889* [UBT]
A. eremastrum Schwartz	22	YEMEN: 20 km E Al Dann	*Mangelsdorff Y25* [ULM]
A. hesperidum (Maire) Plowes	22	MOROCCO: 36 km from Taquist, 1 km above Torres-de-Alcalá	*t'Hart 67-106B* [MSUN]
A. laikipiensis (M. G. Gilbert) Plowes	22	KENYA: 43 km NE Rumuruti	*Newton 4221* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
# *A. laticorona* (M. G. Gilbert) Plowes	22	ETHIOPIA: 2 km S Modjo, SHEWA	*Gilbert s.n. sub Plowes 7695* [MSUN]
# *A. lugardii* (N. E. Br.) Plowes	22	R.S.A.: Northern Cape Prov., 20 km N Bladgrond	*Meve & Liede 613* [MSUN]
A. sacculata (N. E. Br.) Plowes	22	SAUDI ARABIA: s. loc.	*s. coll., s.n.*
# *A. schweinfurthii* (Berger) Plowes	22	TANZANIA: s. loc.	*Speckes 805*
A. semitubiflora L. E. Newton	22	TANZANIA: Kisite Crater	*Newton 3419* [K, EA, MSUN]
# *A. subterranea* (E. A. Bruce & P. R. O. Bally) Plowes	22	KENYA: Mt. Maktau	*Meve et al. 946* [MSUN]
# *A. tubiformis* (E. A. Bruce & P. R. O. Bally) Plowes	22	KENYA: 5 km N Longobito	*Meve, Newton & Goyder 958*
A. vibratilis (E. A. Bruce & P. R. O. Bally) M. G. Gilbert	44	KENYA: 36 km N Horr	*Cumming 1443* [MSUN]
# *A. wissmannii* (O. Schwartz) Plowes	22	OMAN: Sarfait road, 4 km E of turn to Rakhyut	*Jonkers 66* [MSUN]
***Brachystelma* Sims**			
B. barberae Harvey ex Hook. f.	22	ZIMBABWE: W Bulawayo	*Albers 2570* [MSUN]
* *B. bracteolatum* Meve	22	NIGERIA: Jos Plateau	*Specks 549* [K, MSUN]
B. buchananii N. E. Br.	22	TANZANIA: Ruvuma	*Specks 378* [MSUN]
B. burchellii (Decne.) Peckover	22	R.S.A.: Free State, Bloemfontein	*sub Specks 3156* [MSUN, UBT]
B. christianae Peckover	22	R.S.A.: KwaZulu-Natal	*sub Specks 2531* [MSUN]
B. decipiens N. E. Br.	22	R.S.A.: Eastern Cape, Transkei	*sub Specks 2390* [MSUN]
B. filifolium Schltr.	22	s. loc.	*ex hort.* [MSUN]
# *B. gracile* E. A. Bruce	22	ZIMBABWE: Bulawayo, Hyde Park	*Albers 2558*
B. kituloensis Goyder	22	TANZANIA: Makinda, Livingstone Mts.	*Specks 599* [MSUN]
B. lancasteri C. Boele	22	ZIMBABWE: Bulawayo outskirts	*Albers 2573* [MSUN]
B. lineare A. Rich.	22	ETHIOPIA: 27 km SW Bah Dar	*Specks 432* [MSUN]
B. macropetalum N. E. Br.	22	R.S.A.: s. loc.	*sub Specks 2986* [MSUN]
B. megasepalum Peckover	22	TANZANIA: Ruvuma Prov., Kitobaloma	*Specks 748* [MSUN]
B. meyerianum Schltr.	22	R.S.A.: Eastern Cape Prov., Transkei	*sub Specks 2987* [MSUN]
# *B. mortonii* C. C. Walker	22	IVORY COAST (STATE): Comoé-Nationalpark, 6.5 km N Lola Plain	*Porembski sub MS 651* [K, MSUN]
B. nanum N. E. Br.	22	R.S.A.: Mpumalanga	*Peckover sub Specks 2988* [MSUN]
B. remotum R. A. Dyer	22	R.S.A.: Kwazulu-Natal, 45 km SE Wakkerstroom	*Craib s.n.*
B. richardsii Peckover	22	TANZANIA: Ruvuma Prov., Mhukuru	*Specks 762* [MSUN]
B. rubellum (E. Mey.) Peckover	22	R.S.A. Kwazulu-Natal, Thornville, Tala Hill	*Cumming 3565*
B. simplex Schltr.	22	IVORY COAST (STATE): Comoé-Nationalpark, 6.5 km N Lola Plain	*Porembski 8648* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Caralluma* R. Br. s.l.**			
# *C. acutangula* (Decne.) N. E. Br.	22	KENYA: Archer's Post	*Meve 964*
# *C. adenensis* (Deflers) A. Berger	22	OMAN: Wadi Afal	*Jonkers 85* [MSUN]
# *C. adscendens* (Roxb.) R. Br.	22	INDIA: Bangalore, Tahlly	*Bruyns 5937*
# *C. adscendens* var. *attenuata* Wight	22	INDIA: 72 km of Tirumyam direction of Madurai	*Sarkaria J18-90* [MSUN]
# *C. adscendens* var. *fimbriata* (Wall.) Gravely & Mayur.	22	INDIA: Pasarny Ghat Distr., Wai-Mahableshwar Rd., 3–4 km beyond Wai	*Sarkaria 166-77*
C. adscendens var. *geniculata* Gravely & Mayur.	22	INDIA: Marathumalai Hill near Nagercoil	*Sarkaria J30-90* [MSUN]
C. adscendens var. *gracilis* Gravely & Mayur.	22	INDIA: 9 km beyond Tirumykm. near Malai Kuddi Patti	*Sarkaria 62-78*
C. adscendens var. *gracilis* Gravely & Mayur	22/44	INDIA: Yercaud Hill	*Sarkaria J 32-90* [MSUN]
C. arabica N. E. Br.	22	OMAN: Ra's al-Hamra	*Jonkers 16* [ON]
# *C. arachnoidea* (P. R. O. Bally) M. G. Gilbert var. *arachnoidea*	22	KENYA: between Kabernat and Kapengunia	*Hartmann & Newton 28406* [MSUN]
C. baradii Lavranos	22	SOMALIA: 34 km W Hobiyo	*Lavranos et al. 23338* [UPS]
C. bhupinderana Sarkaria	22	INDIA: Palamkottai–Tuticorn Rd., base of Vallandu Hill	*Sarkaria 28-90* [MSUN]
* *C. burchardii* N. E. Br. subsp. *burchardii*	130–132	SPAIN: Canary Islands, Fuerteventura, Betancuria, Bco. de Ajuy	*Wiemers s.n.* [MSUN]
* *C. burchardii* subsp. *maura* (Maire) Meve & F. Albers	66	MOROCCO: 28 km N Sidni Ifni	*Jonkers 223*
# *C. cicatricosa* (Deflers) N. E. Br.	22	YEMEN: Hammam Ali	*Voltee 807* [MSUN]
# *C. crenulata* Wall.	22	INDIA: Bangalore-Kodai road, 2 km beyond Narsapur	*Sarkaria 10-77* [MSUN]
# *C. dalzielii* N. E. Br.	22	CAMEROON: W Mindif, between Maroua and Mindif	*Specks s.n.*
# *C. dicapuae* Chiov. subsp. *dicapuae*	22	KENYA: Longobito	*Meve 955*
# *C. diffusa* (Wight) N. E. Br.	22	INDIA: Tamkur, near Sideshurai Ganga Temple Hill	*Sarkaria 81-75*
# *C. edithae* N. E. Br.	22	ETHIOPIA: S Dire Dawa	*Frank 57* [MSUN]
# *C. edulis* (Edgew.) Benth. & Hook.	22	ex hort. Basel	*s. coll.* [MSUN]
C. edwardsiae (M. G. Gilbert) M. G. Gilbert	22	KENYA: near Turbi	*Hartmann 21393* [MSUN]
# *C. europaea* (Guss.) N. E. Br. var. *europaea*	22	MOROCCO: 55 km from Nador on road to Guercif	*t'Hart 67-33B* [MSUN]
* *C. europaea* (Guss.) N. E. Br. var. *europaea*	22	SPAIN: Almeira, Capo de Gata	*Albers 87-23-004-20* [MSUN]
# *C. europaea* var. *judaica* M. Zohary	22	ISRAEL: Har Hanegev (Jebel Hursha)	*Chaouat s.n.* [HUJ, MSUN]
C. flava N. E. Br.	22	OMAN: Dhofar, Jabal Qamar, on road to Sarfait	*Jonkers 150* [MSUN]
# *C. foetida* E. A. Bruce	22	KENYA: NE Rumuruti, Kisima Ranch	*Meve 950* [UBT]
C. furta P. R. O. Bally	22	DJIBOUTI: s. loc.	*Butler D106* [MSUN]
C. gracilipes K. Schum.	22	TANZANIA: Pare	*Specks 632* [MSUN]
# *C. indica* (Wight & Arn.) N. E. Br.	22	INDIA: 13 km beyond Tiruchirapalli in direction to Puddukottai	*Sarkaria J 11-90*

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
# *C. joannis* Maire	66	MOROCCO: Aoulez, 35 km on the road Taliouine to Agadir	*de Wilde s.n.*
C. lavrani Rauh & Wertel	22	YEMEN: s. loc.	*Frank 119* [MSUN, UBT]
C. moniliformis P. R. O. Bally	22	SOMALIA: 4.5 km N Galgallo	*Koenen & Krapp 22678* [MSUN]
# *C. munbyana* (Decne. ex Munby) N. E. Br.	22	SPAIN: Murcia	*Berthet s.n.* [UBT]
C. pauciflora (Wight) N. E. Br	22	INDIA: Palamkottai	*Bruyns s.n.*
# *C. peckii* P. R. O. Bally	22	s. loc.	*Plowes 7948* [MSUN]
C. priogonium K. Schum.	22	SOMALIA: 26 km N Mogadiscio, ca. 3 km NE Malad	*Koenen & Krapp 49388* [MSUN]
# *C. procumbens* Gravely & Mayur.	22	INDIA: Masuthuamalai Hill, South Travancore	*Sarkaria 108-77*
# *C. sarkariae* Lavranos & Frandsen	22	INDIA: 8 km W Madurai, Madurai-Usilampatti Road, base of Nagamalai hill	*Sarkaria 23-90*
* *C. sinaica* (Decne.) A. Berger	22	ISRAEL: Dead Sea, Dragot shore	*Bruyns 2484* [MSUN]
C. socotrana (Balf. f.) N.E. Br.	22	SOMALIA: 3 km E Los Anod	*Lavranos s.n.*
# *C. speciosa* N. E. Br.	22	KENYA: Archer's Post	*Mere 965*
C. staintonii Hara	22	NEPAL: Jiamiri (Bheri River Valley)	*Bruyns 2515* [MSUN, K]
# *C. stalagmifera* C. E. C. Fischer	22	INDIA: Tirumyam-Madurai, km 72.5	*Sarkaria J17A-90* [MSUN]
# *C. subulata* (Forssk.) Decne.	44	YEMEN: 6 km N Bajil	*Barad 11705*
# *C. tuberculata* N. E. Br.	22	INDIA: 2 km NW Jammu (near Magkola-Kujagh)	*Sarkaria 43-75*
* *C. turneri* E. A. Bruce	22	KENYA: Lake Victoria	*Hartman & Newton 28587* [MSUN]
# *C. umbellata* Haw.	22	INDIA: Tiruchirapalli–Puddukotai road	*Sarkaria 55-77*
***Ceropegia* L.**			
C. africana R. Br. subsp. *africana*	22	R.S.A.: Kzwazulu-Natal Prov., Oribi-Gorge	*Balkwill K 1570*
C. albisepta Jum & H. Perrier	22	MADAGASCAR: betw. Antsirabe and Ambositra (W side of Col des Tapias)	*Lavranos et al. 29964* [MSUN]
C. arabica H. Huber	22	YEMEN: Ussab al Áli	*Mangelsdorff Y26* [UBT]
C. aristolochioides Decne. subsp. *aristolochioides*	22	KENYA: Rift Valley Province, Kajiado District, Alhi River	*Masinde 684* [MSUN]
# *C. aristolochioides* Decne. subsp. *deflersiana* Bruyns	22	YEMEN: Wadi Rima, ca. 35 km W Madinat ash Shirq	*Mangelsdorff Y11* [MSUN]
C. bulbosa Roxb. ("*C. vignaldiana*")	22	ETHIOPIA: Harar, Mt. Achim	*Specks 429* [MSUN]
C. cimiciodora Obermeyer	22	R.S.A.: s. loc.	*ex BG Bonn 2535* [MSUN]
C. crassifolia var. *copleyae* (Bruce & Bally) H. Huber	22	KENYA: Nairobi, near Airport	*Masinde & Meve M 874* [EA, MSUN]
C. cumingiana Decne.	22	PHILIPPINES: Luzon, Mt. Makiling	*Liede 3250* [MSUN]
* *C. dichotoma* Haw. subsp. *dichotoma*	22	SPAIN: Canary Islands, Tenerife, Punta de Teno	*Meve s.n.* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
C. dimorpha Humbert	22	MADAGASCAR: between Ihosy and Ranohira, 10.5 km to Ranohira	*Liede 2638* [MSUN]
C. distincta N. E. Br. ("*C. brevirostre*")	22	TANZANIA: Amani	*Liede & Meve 3379* [EA, UBT]
# *C. elegans* Wall.	22	INDIA: Konanad	*Bruyns 5906* [MSUN]
C. filiformis (Burch.) Schltr.	22	R.S.A.: Northern Cape Prov., Loxton	*Bruyns 6683*
C. gilgiana Werderm.	22	TANZANIA: SW Itigi	*M. & E. Specks 1 039* [MSUN, UBT]
C. hermannii Rauh & M. Teissier	22	MADAGASCAR: Tuléar, E de la Table	*Petignat 455*
C. humbertii H. Huber	22	MADAGASCAR: Montagne de Francais	*Mangelsdorff M 22* [MSUN]
C. intermedia Wight	22	INDIA: Ebanad	*Bruyns 5903*
# *C. juncea* Roxb.	44	INDIA: Tasparc, Vizakapatnam	*Bruyns s.n.*
C. lugardiae N. E. Br.	22	NAMIBIA: Steinhausen, Farm Wunderland	*Grabow s.n. sub As 103*
C. meleagris H. Huber	22	INDIA: s. loc.	*s.n. ex Shirley* [MSUN]
C. meyeri-johannis Engl.	22	KENYA: Mt. Kasigau, SW side	*Meve et al. 936* [K, MSUN]
C. multiflora Bak. subsp. *multiflora*	22	ZIMBABWE: Bulawayo, Hyde Park	*Albers 2559*
# *C. nilotica* Kotschy	22	MALAWI: Njakwa Gorge, S Rukuru River at road from E Kwendeni to Rumpi	*Thiede 2151* [MSUN]
C. occidentalis R. A. Dyer	22	R.S.A.: Western Cape Prov., Strandfontein	*v. Jaarsveld & Bruyns 2321* [MSUN]
C. petignatii Rauh	22	MADAGASCAR: 30 km N Tuléar, near Mora-Mora	*ex Petignat 296* [MSUN]
C. powysii D. V. Field	22	KENYA: Mt. Maktau	*Meve 941* [K, MSUN]
C. racemosa N. E. Br. susbp. *glabra* H. Huber	44	MADAGASCAR: Ankarana	*Mangelsdorff M 27* [MSUN]
C. racemosa N. E. Br. subsp. *racemosa*	22	TANZANIA: Kilimanjaro, Lake Chala	*Meve & Liede 3355* [EA, UBT]
# *C. rupicola* Deflers	22	YEMEN: Djebel Haraz	*Mangelsdorff Y 13* [MSUN]
C. sankuruensis Schltr.	22	CAMEROON: Mt Cameroon, W Likombe	*Meve 909* [K, MSUN]
C. saxatilis Jum. & H. Perrier	22	MADAGASCAR: Ankarana Mts., Montagne de Francais	*Mangelsdorff s.n.* [MSUN]
C. simoneae Rauh	22	MADAGASCAR: forest between Tishombé and Ihado	*Rauh 73117* [HEID, MSUN]
C. stapeliaeformis Haw.	22	s. loc.	*s.n. ex Palmengarten*
C. stentiae E. A. Bruce	22	R.S.A.: North-West Prov., NW Potchefstroom	*Albers et al. 3529* [MSUN]
* *C. striata* Meve & Masinde	22	MADAGASCAR: Antsirabe	*Rauh 75007* [HEID, MSUN]
C. subaphylla K. Schum.	22	SAUDI ARABIA: Muhayl	*Collenette 7307* [MSUN]
# *C. thwaitesii* Hook.	22	INDIA: Davikolam	*Bruyns 5944*
# *C. variegata* Decne.	22	KENYA: 3 km N Maralal	*Masinde & Meve 871* [MSUN]
***Duvalia* Haw.**			
* *D. angustiloba* N. E. Br.	22	R.S.A: Western Cape Prov., 82 km E Beaufort West	*Meve 319* [MSUN, NBG]
* *D. caespitosa* (Mass.) Haw. var. *caespitosa*	44	R.S.A: Western Cape Prov., betw. Muiskraal and Barrydale, nr Kleindoornrivier	*Albers & Meve 129* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
* *D. caespitosa* (Mass.) Haw. var. *compacta* (Haw.) Meve	44	R.S.A: Western Cape Prov., E VanRhynsdorp	*Meve 263* [MSUN]
* *D. elegans* (Mass.) Haw.	22	R.S.A: Western Cape Prov., 7.5 km N McGregor	*Meve 383* [MSUN]
* *D. immaculata* (Lückh.) Bayer ex L. C. Leach	66	R.S.A: Western Cape Prov., Gouritzmond	*Meve 401* [MSUN]
# *D. maculata* N. E. Br.	22	R.S.A: Western Cape Prov., Sterkfontein	*Bruyns 6289*
# *D. sulcata* N. E. Br. subsp. *sulcata*	44	YEMEN: Lodar	*Hanacek 286* [in hort. UBT]
D. sulcata N. E. Br. subsp. *somalensis* (Lavranos) Meve	22	DJIBOUTI: s. loc.	*Butler D105*
***Duvaliandra* M. G. Gilbert**			
# *D. dioscorides* (Lavranos) M. G. Gilbert	22	YEMEN: Socotra, Hadiboh	*Orlando s.n.* [UBT]
***Echidnopsis* Hook. f.**			
E. angustiloba E. A. Bruce & P. R. O. Bally	22	KENYA: ca. 5 km N Longobito	*Meve 959* [K, MSUN, UBT]
E. archeri P. R. O. Bally	22	KENYA: Nguruman Escarpment, Oloibitato River near Hayton's Falls	*Archer in Bally S325* [K, ZSS]
E. ballyi (M. Lapostolle) P. R. O. Bally	22	SOMALIA: s. loc.	*Plowes 6609*
E. bihendulensis P. R. O. Bally	22	SOMALIA: below the Sheik Pass at Bihendula	*Reynolds 1956*
E. cereiformis Hook. f.	22	SUDAN: Erkovit, Jebel Aulini	*Hartmann 21505* [MSUN]
# *E. dammaniana* Sprenger	22	KENYA: S Gatap	*Hartmann & Newton 28557* [MSUN]
E. globosa Thulin & Hjertson	22	YEMEN: Hadramaut, 11 km from the turning to the Masila field	*Thulin et al. 8248* [K, UPS]
# *E. leachii* Lavranos	22	TANZANIA: Dodoma	*Specks 1264* [UBT]
# *E. malum* (Lavranos) Bruyns	22	SOMALIA: 40 km NE Mogadishu	*Lavranos 23224A*
# *E. mijerteina* Lavranos	22	SOMALIA: 2 km S Eil	*Lavranos 8446*
# *E. repens* R. A. Dyer & Verd.	22	KENYA: Mt. Maktau	*Meve 942*
E. scutellata subsp. *australis* Bruyns	22	KENYA: Marsabit, N side of Gof Chjoba crater	*Lavranos & Bleck 19527*
E. scutellata subsp. *dhofarensis* Bruyns	22	OMAN: Dhofar Province, on road from Thamarit to Salalah, Agabat al Hatab	*Miller 2811* [E]
# *E. scutellata* subsp. *planiflora* (P. R. O. Bally) Bruyns	22	ETHIOPIA: Jijiga, Marda Pass	*Gilbert s.n.* sub *Plowes 4264*
E. scutellata subsp. *planiflora* (P. R. O. Bally) Bruyns	44	SOMALIA: 4 km NW Erigavo	*Lavranos 7325* [FT]
# *E. scutellata* (Deflers) A. Berger subsp. *scutellata*	22	YEMEN: road from Kuhlan to Hajjah	*Miller & Long 3259* [E]
E. sharpei subsp. *ciliata* (P. R. O. Bally) Bruyns	22	SOMALIA: Sheik Pass	*Bailes 134* [MSUN]
E. sharpei A. C. White & B. Sloane subsp. *sharpei*	22	KENYA: S Moyale	*Hartmann & Newton 21381* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
# *E. squamulata* (Decne.) P. R. O. Bally	44	YEMEN: Amiri Highlands, 6 km NE Dhala	*Lavranos 1869* [K, PRE]
E. urceolata P. R. O. Bally	22	KENYA: s. loc.	*Lavranos 12220*
E. urceolata P. R. O. Bally	44	YEMEN: 15 km S Taizz (Aden road)	*Lavranos & Newton 13101*
E. virchowii K. Schum.	22	SOMALIA: s. loc.	*Bailes sub Plowes 6610* [MSUN]
# *E. watsonii* P. R. O. Bally	44	SOMALIA: 8 km W Bawn	*Lavranos 10421* [MSUN]
E. yemenensis Plowes	22	YEMEN: base of Menakha Pass near Suq al Khamis	*Plowes & Barad 7764* [SRGH]
***Edithcolea* N. E. Br.**			
E. grandis N. E. Br.	22	KENYA: Ndi Area	*Meve 932*
***Heterostemma* Wight & Arn.**			
H. acuminatum Decne.	22	AUSTRALIA: Port Curtis Distr., Olsens Caves	*Forster 5090* [BRI, K, L, MEL, MO]
H. herbertii Elmer	22	PHILIPPINES: Luzon, Mt Makiling	*Liede 3271b* [UBT]
***Hoodia* Sweet ex Decne.**			
# *H. gordonii* (Masson) Sweet	22	NAMIBIA: ca. 30 km S Warmbad, Farm Eendoorn (Pretorius)	*Meve & Struck 192* [MSUN]
H. juttae Dinter	22	NAMIBIA: Vredenhof	*Bruyns 3508* [MSUN]
H. longispina Plowes	22	NAMIBIA: Witpütz	*Albers & Meve 85* [MSUN]
H. officinalis (N. E. Br.) Plowes subsp. *delaetiana* (Dinter) Bruyns	22	NAMIBIA: Klinghardt	*Hammer 914*
H. pedicellata (Schinz) Plowes	22	NAMIBIA: Rössingberge	*Albers 2718* [MSUN]
H. pillansii N. E. Br.	22	R.S.A.: s. loc.	*s. coll. sub Al 2510*
H. pilifera (L. f.) Plowes subsp. *pilifera*	22	R.S.A.: Western Cape Prov., ca. 19 km SE Oudtshoorn	*Meve 406* [MSUN]
# *H. ruschii* Dinter	22	NAMIBIA: Uri Hauchab	*Jürgens 91245*
***Huernia* R. Br.**			
H. arabica N. E. Br.	22	YEMEN: 3 km N Ar Rahida	*Barad 11752* [MSUN]
H. archeri L. C. Leach	44	KENYA: 100 km W Malindi, Galana River, Lali Hill	*Powys 804*
# *H. aspera* N. E. Br.	44	KENYA: Mt Maktau	*Meve 943b* [MSUN]
H. boleana M. G. Gilbert	22	ETHIOPIA: N Addis Ababa, Bole Valley	*Horwood 11072* [MSUN]
H. brevirostris N. E. Br. subsp. *brevirostris*	22	R.S.A.: Eastern Cape Prov., Steytlerville, Springbokvlakte	*Lavranos & Pehlemann 19868*
H. erinacea P. R. O. Bally	44	KENYA: between Moyale and Marsabit, Sololo hill	*Cumming 1416*
# *H. guttata* (Masson) Haw.	22	R.S.A.: Western Cape Prov., near Calitzdorp Dam	*Leach & Rossouw 16147* [MSUN, NBG]
# *H. hallii* E. Lamb & B. M. Lamb	22	NAMIBIA: ca. 29 km E Aus, Farm Plateau	*Meve & Struck 181* [MSUN]
# *H. humilis* (Masson) Haw.	22	R.S.A.: Eastern Cape Prov., Fraserburg, Tafelberg	*LCL 17710*
# *H. keniensis* R. E. Fries	44	KENYA: 30 km N Maralal	*Meve 963* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
# *H. keniensis* R. E. Fries var. *keniensis*	44	KENYA: Tugen Hills	*Newton 4283*
H. keniensis var. *nairobiensis* A. C. White & B. Sloane	22	KENYA: 4 km N Narock	*Cumming 1032* [MSUN]
H. lenewtonii Plowes	22	KENYA: E Lake Turkana, Sul-Sul Mudde	*Newton & Powys 3703* [EA, K]
# *H. nigeriana* Lavranos	22	NIGERIA: s. loc.	*Plowes 7856* [MSUN]
H. pendula E. A. Bruce	22	R.S.A.: Eastern Cape Prov., Transkei, s. loc.	*s. coll*
# *H. penzigii* N. E. Br.	22	YEMEN: Taizz, 15 km NE Airport	*Plowes 7803* [MSUN]
# *H. procumbens* (R. A. Dyer) L. C. Leach	44	R.S.A.: Mpumalanga, Masisi Village	*Hardy 5404* [NBG]
# *H. reticulata* (Masson) Haw.	22	R.S.A.: Western Cape Prov., VanRhynsdorp, Giftberg	*Lavranos 28207*
H. rosea Newton & Lavranos	22	YEMEN: 5 km W Al Magrabah	*Noltee 1528* [MSUN]
H. rubra Plowes	22	YEMEN: Ramadah	*Noltee 2092* [MSUN]
# *H. saudi-arabica* D. V. Field	22	YEMEN: Taifa/Hadda	*Lavranos & Collenette 18467* [MSUN]
H. thudichumii L. C. Leach	22	R.S.A.: Eastern Cape Prov., s. loc.	*Albers 2521* [MSUN]
# *H. verekeri* Stent var. *verekeri*	22	ZIMBABWE: NW Hwange, "Thomson Junction"	*Albers, Liede & Meve 523* [MSUN]
# *H. volkartii* Peitsch. ex Werderm. & Peitsch. var. *volkartii*	44	ZIMBABWE: Buchwa, Old Iron Mine (Masvingo Distr.)	*Erwee s.n. sub Albers 2574*
H. zebrina N. E. Br. subsp. *magniflora* (Phillips) L. C. Leach	22	R.S.A.: Northern Prov., Zaaiplaats bei Potgietersrust, Tin-Mine	*Balkwill K 1567*
# *H. zebrina* n. E. Br. subsp. *zebrina*	22	R.S.A.: 23°23′S, 29°45′E	
***Huerniopsis* R. Br.**			
* *H. decipiens* N. E. Br.	22	R.S.A.: Northern Cape Prov., 11.4 km E Kuruman	*Meve & Liede 580* [MSUN]
***Larryleachia* Plowes**			
# *L. cactiformis* (Hook.) Bruyns	22	NAMIBIA: s. loc.	*Albers 2513* [MSUN]
* *L. marlothii* (N. E. Br.) Bruyns	22	NAMIBIA: 8 km S Rosh Pinah	*Albers & Meve 45* [MSUN]
* *L. picta* (N. E. Br.) Bruyns subsp. *picta*	22	NAMIBIA: ca. 29 km E Aus, Farm Plateau	*Meve & Struck 178* [MSUN]
***Lavrania* Plowes**			
L. haagnerae Plowes	22	NAMIBIA: 40–50 km SE Sesfontein, Khowarib Gorge	*Haagner sub Plowes 5046* [PRE]
***Leptadenia* R. Br.**			
L. pyrotechnica Decne.	22	EGYPT: 24 km W Safaga	*Albers 3643* [MSUN]
L. hastata Decne.	22	GAMBIA: Abouko Natl. Park	*S. Huber s.n.*
***Neoschumannia* Schltr.**			
N. cardinea (S. Moore) Meve	22	TANZANIA: E Usambara, Amani Forest Reserve	*Meve & Liede 3359* [B, UBT]
* *N. kamerunensis* Schltr.	22	CAMEROON: Mt. Cameroon, NW Likombe	*Meve & Etuge 910* [B, K, MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Notechidnopsis* Lavranos & Bleck**			
* *N. tessellata* (Pillans) Lavranos & Bleck	22	R.S.A.: Northern Cape Prov., ca. 13 km N Nieuwoudtville, "Uithoek"	*Meve 255* [MSUN]
***Ophionella* Bruyns**			
# *O. arcuata* (N. E. Br.) Bruyns subsp. *arcuata*	22	R.S.A: Eastern Cape Prov.	*Bruyns 4966* [MSUN, UBT]
***Orbea* Haw.**			
O. irroata (Masson) L. C. Leach	22	s. loc.	*s. coll.*
# *O. longidens* (N. E. Br.) L. C. Leach	44	R.S.A: Kwazulu-Natal, Lower Mt. Kergl	*Hardy 6255*
# *O. miscella* (N. E. Br.) Meve	22	R.S.A.: Eastern Cape Prov., Richmond	*Bruyns 3237* [MSUN]
# *O. namaquensis* (N. E. Br.) L. C. Leach	22	R.S.A.: Northern Cape Prov., 4 km N Steinkopf	*Albers & Meve 111* [MSUN]
O. paradoxa (Verd.) L. C. Leach	44	s. loc.	*s. coll.* [MSUN]
O. rangeana (Dinter & A. Berger) L. C. Leach	22	NAMIBIA: 1 km N Klein Karas	*Albers 2356a* [MSUN]
# *O. semota* (N. E. Br.) L. C. Leach	44	TANZANIA: s. loc.	*Specks 921* [MSUN]
# *O. tapscottii* (Verd.) L. C. Leach	22	BOTSWANA: 15 km NW Molepolole	*Plowes 7829*
# *O. variegata* (L.) L. C. Leach	22	R.S.A.: Western Cape Prov., St. Helena Bay	*Bruyns 4571* [MSUN]
# *O. variegata* (L.) L. C. Leach	44	R.S.A.: Eastern Cape Prov., Stytlerville	*Albers 2466* [MSUN]
# *O. verrucosa* (N. E. Br.) L. C. Leach var. *verrucosa*	22	R.S.A.: Eastern Cape Prov., Grahamstown, Thomas Baines N. R. nr Damwall	*Liede 2919* [MSUN]
***Orbeopsis* L. C. Leach**			
# *O. albocastanea* (Marl.) L. C. Leach	22	NAMIBIA: s. loc.	*Bruyns 3045*
* *O. gerstneri* subsp. *elongata* (R. A. Dyer) L. C. Leach	22	R.S.A.: Mpumalanga, Pilgrim's Rest, Penge	*Hardy 4095*
O. gerstneri (Letty) L. C. Leach subsp. *gerstneri*	44	R.S.A.: Kwazulu-Natal, Empangeni	*de Kock sub Albers 2551* [MSUN]
# *O. lutea* (N. E. Br.) L. C. Leach subsp. *lutea*	22	R.S.A.: Northern Cape Prov., 15 km W Potchefstrom	*Albers 3525* [in hort. UBT]
# *O. lutea* (N. E. Br.) L. C. Leach subsp. *lutea*	22	ZIMBABWE: Bulawayo, Hyde Park	*Albers, Liede & Meve 537* [MSUN]
# *O. melanantha* (Schltr.) L. C. Leach	44	ZIMBABWE: Zambezi Valley, between Deka River & Binga River	*Plowes 6502*
O. tsumebensis (Oberm.) L. C. Leach	22	NAMIBIA: 5 km N Grootfontein	*Rawe s.n.* [MSUN]
* *O. valida* (N. E. Br.) L. C. Leach	22	ZIMBABWE: Wankie Distr., Kamativi	*Buckland s.n.*
***Orthanthera* Wight**			
# *O. albida* Schinz	22	NAMIBIA: near the Groot Welwitschia Vlakte	*Albers 2727* [MSUN]
***Pachycymbium* L. C. Leach**			
* *P. carnosum* (Stent) L. C. Leach	22	R.S.A.: Northern Cape Prov., Magaliesberg, Breedts Nek	*Hardy sub Plowes 6715* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Pectinaria* Haw.**			
# *P. longipes* (N. E. Br.) Bruyns	22	R.S.A.: Western Cape Prov., Keerom, Sutherland	*Bruyns 2548* [MSUN]
***Piaranthus* R. Br.**			
* *P. comptus* N. E. Br.	22	R.S.A.: Western Cape Prov., between Klaarstrom and Prince Albert	*Albers et al. 10708 in K 1123* [MSUN]
* *P. decorus* (Masson) N. E. Br. subsp. *decorus*	22	R.S.A.: Western Cape Prov., near Sterkfontein	*Bruyns 6290*
* *P. parvulus* N. E. Br.	22	R.S.A: Western Cape Prov., ca. 7 km SW Laingsburg (Witteberge)	*Meve 288* [MSUN]
***Pseudolithos* P. R. O. Bally**			
P. caput-viperae Lavranos	22	*ex hort.* Mesa Garden	*s.n.* [MSUN]
* *P. dodsonianus* (Lavranos) Bruyns & Meve	22	SOMALIA: 1 km NNW Erigavo	*Lavranos 7326* [MSUN]
***Quaqua* N. R. Br.**			
# *Q. acutiloba* (N. E. Br.) Bruyns	22	R.S.A.: Northern Cape Prov., 11 km N Concordia	*Meve 213* [MSUN]
Q. albersii Plowes	22	R.S.A: Western Cape Prov., bet. Vredendal and Holrivier to Liebendal	Albers 2252 [MSUN]
# *Q. cincta* (Lückh.) Bruyns	22	R.S.A.: Northern Cape Prov., N Concordia	*Pehlemann 2737* [MSUN]
# *Q. incarnata* subsp. *aurea* (Lückh.) Bruyns	22	R.S.A: Western Cape Prov., Pakhuis Pass	*Albers 2031b* [MSUN]
# *Q. incarnata* (L. f.) Bruyns subsp. *incarnata* var. *incarnata*	22	NAMIBIA: N Aus, branching off Helmeringhausen	*Albers & Meve 95* [MSUN]
Q. incarnata subsp. *incarnata* var. *tentaculata* Bruyns	22	R.S.A: Western Cape Prov., 40 km N Nieuwoudtville, Kokerboomkop	*Meve 246* [MSUN]
Q. inversa N. E. Br.	22	R.S.A: Western Cape Prov., Bitterfontein	*Bruyns 6133* [MSUN]
Q. linearis (N. E. Br.) Bruyns	22	R.S.A: Western Cape Prov., Spreenfontein	*Bruyns 2423* [MSUN]
# *Q. mammillaris* (L.) Bruyns	22	R.S.A: Western Cape Prov., 15 km S VanRhynsdorp	*Meve 146* [MSUN]
# *Q. marlothii* (N. E. Br.) Bruyns	22	R.S.A: Western Cape Prov., 1 km N Karoopoort	*Albers et al. K1415* [MSUN]
Q. multiflora N. E. Br.	22	R.S.A: Northern Cape Prov., Calvinia	*Bruyns 4297* [MSUN]
Q. parviflora (Masson) Bruyns subsp. *gracilis* (Lückh.) Bruyns	22	R.S.A: Western Cape Prov., below Gannaga Pass	*Bruyns 989* [MSUN]
Q. parviflora (Masson) Bruyns subsp. *pulchra* Bruyns	22	R.S.A: Western Cape Prov., between Vredendal and Holrivier	*Albers 2253*
Q. parviflora (Masson) Bruyns subsp. *swanepoelii* (Lavr.) Bruyns	22	R.S.A.: Northern Cape Prov., 30 km S Loerisfontein on road to Calvinia	*Lavranos s.n.* [MSUN]
Q. pillansii (N. E. Br.) Bruyns	22	R.S.A: Western Cape Prov., Montagu, Kogmanskloof	*EH 190 sub Albers 2553*
Q. pruinosa (Masson) Bruyns	22	s. loc.	*ex Kennedy sub Albers 2514* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
Q. ramosa (Masson) Bruyns	22	R.S.A: Western Cape Prov., SW Laingsburg	*Albers 2481* [MSUN]
***Rhytidocaulon* P. R. O. Bally**			
R. fulleri Lavranos & Mort.	22	OMAN: Dhofar, 56 km E Salalah	*Collenette 8439* [MSUN]
* *R. macrolobum* Lavranos subsp. *minumum* Meve & Collenette	22	SAUDI ARABIA: 60 km NW Najran	*Collenette 8792* [MSUN]
R. sheilae D. V. Field	22	SAUDI ARABIA: Muhayl	*Collenette 8964*
***Riocreuxia* Decne.**			
R. burchellii K. Schum.	22	R.S.A.: Kwazulu-Natal, s. loc.	*ex hort. Shirley* [MSUN]
***Stapelia* L.**			
# *S. asterias* Masson	22	R.S.A: Western Cape Prov., between Calitzdorp and Van-Wyksdorp	*Albers et al. 10726 in K 1136* [MSUN]
S. baylissii L. C. Leach	22	R.S.A.: Eastern Cape Prov., Zuurberg Pass	*Bayer s.n.* [MSUN]
S. erectiflora subsp. *prostratiflora* L. C. Leach	22	R.S.A: Western Cape Prov., Dornrivier Bridge	*Bruyns 1289* [NBG]
# *S. gigantea* N. E. Br.	22	MALAWI: Lake Malawi	*Albers 2575*
S. glanduliflora Masson	22	R.S.A: Western Cape Prov., 25 km S Klawer	*Albers & Meve 04* [MSUN]
# *S. grandiflora* Masson	22	LESOTHO: NW Mafeteng, Hermon	*Gerbaulet & Struck 23783* [MSUN]
# *S. hirsuta* L.	22	R.S.A: Western Cape Prov., Veldrif	*Albers 2498* [MSUN]
# *S. kwebensis* N. E. Br.	22	ZIMBABWE: Tuli, at Botswana border	*Albers 2568* [MSUN]
S. longipedicellata (A. Berger) N. E. Br.	22	NAMIBIA: W Khorixas	*Marschewski 114* [MSUN]
S. montana L. C. Leach var. *montana*	22	R.S.A: Western Cape Prov., Groot Winterhoekberge, near Pardevlei	*Bayer 1602* [MSUN, NBG]
# *S. pillansii* N. E. Br. var. *pillansii*	22	R.S.A: Western Cape Prov., S. Palmietfontein, "Jagerskraal"	*Meve 299*
S. remota R. A. Dyer	22	NAMIBIA: Baynes Mtns. (Kaokaoveld)	*Steenkamp sub Plowes 5146*
# *S. rufa* Masson	22	R.S.A: Western Cape Prov., 27 km S Laingsburg	*Albers 2483* [MSUN]
S. schinzii A. Berger & Schltr. var. *schinzii*	22	NAMIBIA: W Windhoek	*Pehlemann 445*
***Stapelianthus* Choux ex A. C. White & B. Sloane**			
S. arenarius Bosser & Morat	22	MADAGASCAR: N Tulear, Morombe road	*Teissier 111* [MSUN]
S. decaryi Choux	22	MADAGASCAR: Beloha direction to Tsihomb, 4 km from Tsiomb	*Liede, Conrad & Barad 2747* [MSUN]
S. hardyi Lavranos	22	MADAGASCAR: s. loc.	*ex hort. de Boer* [MSUN]
# *S. madagascariensis* (Choux) Choux	22	MADAGASCAR: Ambovombé direction to Antanimora, 15 km from Antanimora	*Liede, Conrad & Barad 2788* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
S. montagnacii (Boit.) Boit. & Bertrand	22	MADAGASCAR: s. loc.	*s.n.* [MSUN]
S. pilosus Lavranos & Hardy	22	MADAGASCAR: Amboasary (S)	*Mies & Nober 51b*
***Stapeliopsis* Pill. (= *Pectinaria* Haw. p.p.)**			
S. breviloba (R. A. Dyer) Bruyns	22	s. loc.	*ex hort. De Boer* [MSUN]
# *S. exasperata* (Bruyns) Bruyns	22	R.S.A.: Northern Cape Prov., Middlepos, Wolvedans	*Bruyns 4513* [MSUN]
# *S. pillansii* (N. E. Br.) Bruyns	22	R.S.A.: Eastern Cape Prov., Port Elizabeth, NW von Ann's Villa	*Bruyns 1561*
# *S. saxatilis* (N. E. Br.) Bruyns subsp. *saxatilis*	22	R.S.A.: Western Cape Prov., 19 km SE Oudtshoorn	*Meve 410* [MSUN]
***Tavaresia* Welw.**			
# *T. barklyi* (Dyer) N. E. Br.	22	NAMIBIA: Kaokoveld	*Albers 3599* [MSUN]
***Tridentea* Haw.**			
T. dwequensis (Lückh.) L. C. Leach	22	R.S.A.: Northern Cape Prov., 45 km ESE Brandvlei	*Karoo Garden 117/82* [MSUN]
# *T. gemmiflora* (Masson) Haw.	22	R.S.A.: Western Cape Prov., 13 km E Murraysburg	*Meve 348* [MSUN]
# *T. jucunda* var. *cincta* (Marl.) L. C. Leach	22	R.S.A.: Eastern Cape Prov., 15 km S Hutchinson	*Meve 349* [MSUN]
# *T. marientalensis* (Nel) Leach subsp. *albipilosa* (Giess) Leach	22	NAMIBIA: Karasburg, Farm Elandsdraai (WSW Ariamsvlei)	*Albers 2348* [MSUN]
# *T. pachyrrhiza* (Dinter) L. C. Leach	22	R.S.A.: Northern Cape Prov., Richtersveld, Oranjemund	*Hardy 4739*
T. parvipuncta var. *truncata* (Lückh.) L. C. Leach	22	R.S.A.: Western Cape Prov., Botterkloof Pass	*Vorster & Lavranos 2994* [MSUN]
T. virescens (N. E. Br.) L. C. Leach	22	R.S.A.: Northern Cape, N Brandvlei	*Lamberti s.n.* [in cult. UBT]
***Tromotriche* Haw.**			
# *T. aperta* (Masson) Sweet	22	R.S.A.: Richtersveld	*Visser sub Albers 2498* [MSUN]
# *T. baylissii* (L. C. Leach) Bruyns var. *baylissii*	22	R.S.A.: Eastern Cape Prov., Kouga Dam	*v. Jaarsveld 11057* [MSUN]
# *T. choanantha* (Lavranos & Hall) Bruyns	22	R.S.A.: Eastern Cape Prov., Kleinberg	*v. Jaarsveld 684/87* [MSUN]
# *T. longii* (Lückh.) Bruyns	22	R.S.A.: Eastern Cape Prov., 55 km SE Jansenville, Pardepoort	*Albers 2464* [MSUN]
* *T. longipes* (Lückh.) Bruyns	22	NAMIBIA: ca. 16 km N Rosh Pinah	*Albers & Meve 75* [MSUN]
# *T. revoluta* (Masson) Haw.	22+2B	R.S.A.: Western Cape Prov., 9 km S Botterkloof Pass	*Albers 2048a* [MSUN]
T. ruschiana (Dinter) Bruyns	22	*s. loc.*	*cult. J. Thompson*
***White-sloanea* Chiov.**			
W. crassa (N. E. Br.) Chiov.	22	SOMALIA: between Garawe and Gardo, Dan Garayo	*Lavranos & Barad s.n.*

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
Tribe: ASCLEPIADEAE (R. Br.) Duby			
Subtribe: Asclepiadinae Endl. ex Meisn.			
***Asclepias* L.**			
# *A. fascicularis* Decne.	22	U.S.A.: *ex hort.* Davis	*ex hort. Davis* [UBT]
A. flava N. E. Br.	22	ARGENTINA: San Pedro de Colalao (Tucumán)	*Liede 3096*
# *A. linaria* Cav.	22	MEXICO: Michoacán, 3 km NW Los Reyes and 25 km E Maravatio	*Conrad 9305* [MSUN]
A. tuberosa L.	22	U.S.A.: ex hort BG Michigan	*ex hort. BG Michigan*
***Calotropis* R. Br.**			
C. gigantea (L.) R. Br.	22	TANZANIA: Amboni	*Liede & Meve 3378* [UBT]
C. procera (Ait.) Ait. f.	22	KENYA: Rift Valley to Baringo, W Koriema	*Liede & Newton 3177*
***Gomphocarpus* R. Br.**			
G. cancellatus (Burm. f.) Bruyns	22	R.S.A.: Western Cape Prov., Rooinekpass	*Albers 3031* [MSUN]
G. filiformis (E. Mey.) Dietr.	22	NAMIBIA: near Groot Welwitschia Vlakte	*Albers 2726* [MSUN]
G. linearis D. Dieter	22	KENYA: Naivasha, Nakuru-Nairobi (Rift Valley)	*Liede 3180* [ULM]
G. tenuifolius (N. E. Br.) Bullock	22	ZIMABAWE: Mtoko	*Albers, Liede & Meve 511* [MSUN]
***Kanahia* R. Br.**			
K. laniflora (Forssk.) R. Br. s.l.	22	KENYA: Teita, Sala Gate, (Tsavo East)	*Liede & Newton 3211* [E, MSUN, ULM]
K. laniflora (Forssk.) R. Br. s. str.	22	YEMEN: North	*Mangelsdorff Y116* [UBT]
***Pergularia* L.**			
# *P. daemia* (Forssk.) Chiov. var. *daemia*	22	CAMEROON: Rhumsiki, centre of village	*Meve 920* [MSUN]
# *P. daemia* (Forssk.) Chiov. var. *daemia*	22	TANZANIA: Moshi, Rau-Forest	*Liede & Meve 3367* [UBT]
P. daemia (Forssk.) Chiov. var. *macrantha* Chiov.	22	KENYA: 56 km W Malindi	*Liede & Newton 3209* [ULM]
P. gariepensis N. E. Br.	22	R.S.A.: Northern Cape Prov., Richtersveld	*Albers 3579* [MSUN]
P. tomentosa L.	22	YEMEN: 10 km W Madinet as Sharq	*Mangelsdorff Y213* [UBT]
***Stathmostelma* K. Schum.**			
S. diversifolia Goyder	22	KENYA: Narok, ca. 32.2 km on Kikorok Road, Loita Plains	*Liede & Newton 3221* [K]
***Stenostelma* Schltr.**			
S. capense Schltr.	22	R.S.A.: Eastern Cape Prov., Teviot Stn.	*Liede 2934* [K, MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
Subtribe: Astephaninae Endl. ex Meisn.			
***Microloma* R. Br.**			
* *M. incanum* Decne.	20	NAMIBIA: 5 km N Aus	*Albers & Meve 88* [MSUN]
* *M. sagittatum* (L.) R. Br.	20	R.S.A.: Western Cape Prov., 22 km N Matjiesfontein	*Meve 376a*
* *M. tenuifolium* (L.) K. Schum.	20	R.S.A.: Western Cape, Kapokberg	*Meve & Liede 623* [MSUN]
Subtribe: Glossonematinae K. Schum.			
***Glossonema* Decne.**			
G. boveanum (Decne.) Decne. ssp. *boveanum*	22	KENYA: ca. 30 km from Loyengalami	*Liede & Vencton 3239* [UBT, ULM]
***Pentarrhinum* E. Mey.**			
# *P. insipidum* E. Mey.	22	R.S.A.: Gauteng, Irene-Verwoerdburg	*Liede 2941* [MSUN]
P. insipidum E. Mey.	24	KENYA: Isiolo	*Liede & Vencton 3225*
Subtribe: Gonolobinae (G. Don) Liede			
***Fischeria* DC.**			
F. sp.	22	COSTA RICA: Region Guana Caste, Cabo Blanco	*Voigt s.n.* [in cult. UBT]
***Gonolobus* Michx.**			
G. barbatus HBK	22	MEXICO: Jalisco, 2 km NE Tequila	*Conrad 9325* [UBT]
***Macroscepis* Kunth**			
M.sp. (near urceolata Karst.)	22	GUATEMALA: 60 km N Guatemala-City	*Heyne s.n.* [UBT]
***Matelea* Aubl.**			
M. carolinensis (Jacq.) R. E. Woodson	22	U.S.A.: Georgia, Athens	*Lipow s.n.*
M. cyclophylla (Standl.) R. E. Woodson	22	MEXICO: s. loc.	*ex hort. G. Barad*
Subtribe: Metastelminae Endl. ex Meisn.			
***Blepharodon* Decne.**			
B. salicinum Decne.	22	ECUADOR: betw. Estación Scientifica San Francisco and Zamora	*Liede 3304* [ULM]
***Cynanchum* L.**			
C. abyssinicum Decne.	22	TANZANIA: Arusha Prov., Olmoti Crater	*Liede & Meve 3373* [UBT]
C. ampanihense Jum. & H. Perrier	22	MADAGASCAR: Cap St. Marie	*Liede 2817a* [MSUN]
C. andringitrense Choux	22	MADAGASCAR: s. loc.	*Rauh s.n.* [MSUN]
C. angavokeliense Choux	22	MADAGASCAR: s. loc.	*s.n. ex Specks* [MSUN]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
C. appendiculatopsis Liede	22	MADAGASCAR: s. loc.	*Fervier s.n.* [MSUN]
C. arenarium Jum. & H. Perrier	22	MADAGASCAR: Tuléar, 15 km to Antanimora	*Liede 2789*
C. auriculatum Royle ex Wight	22	BHUTAN: Pass from Thimpu to Wangduephodrang	*Liede 3281* [MSUN]
C. bisinuatum Jum. & H. Perrier	22	MADAGASCAR: Toliara	*Decoings 28283* [UBT]
C. boerhaviifolium Hook. & Arn.	22	CHILE: ca. 2 km N Los Vilos	*Liede 3062*
C. compactum Rauh	22	MADAGASCAR: Fiananrantsoa, ca. 42 km W Ambatofinadrahana	*Supthut & v. Arx 3029*
C. cucullatum N. E. Br.	22	MADAGASCAR: Ankaratra	*Liede 2868* [MSUN]
C. descoingsii Rauh	22	MADAGASCAR: Tuléar	*Descoings 28244*
C. erythranthum Jum. & H. Perrier	22	MADAGASCAR: Ankarana	*Mangelsdorff M 26*
C. danguyanum Choux	22	MADAGASCAR: Maintirano	*Mangelsdorff 44* [UBT]
C. formosum N. E. Br.	22	CHILE: Los Vilos, ca. 2 km from town	*Liede & Conrad 3061* [MSUN, UBT]
# *C. gerradii* (Harv.) Liede	22	KENYA: 30 km N Maralal	*Meve 962* [MSUN]
C. gerradii (Harv.) Liede	22	MADAGASCAR: betw. Ambovombé and Antanimora, sidetrack to Angavo	*Liede 2797* [MSUN]
# *C. hardyi* Liede & Meve	22	MADAGASCAR: Ankarafansika	*Mangelsdorff M37*
C. implicatum Jum. & H. Perrier	22	MADAGASCAR: Montagne des Francais	*Mangelsdorff 24*
C. juliani-marnieri Descoings	22	MADAGASCAR: Cap St. Marie	*ex hort. Les Cèdres* [MSUN]
C. laeve Pers.	22	U.S.A.: Missouri, St. Louis, near Shaw Arboretum	*Liede s.n.* [UBT]
C. lecomtei Choux	22	MADAGASCAR: Cap St. Marie	*Liede 2817b* [MSUN]
C. leucanthum K. Schum. subsp. *elongatum* Liede	22	MADAGASCAR: 48 km W Samabava	*Mangelsdorff 410* [UBT]
C. madagascariense K. Schum.	22	MADAGASCAR: forest 43 km from Ft. Dauphin	*Liede 2756* [MSUN]
C. marnieranum Rauh	22	MADAGASCAR: 43 km from Ambovombé direction to Tsihombé	*Liede 2805* [MSUN]
C. messeri (Buchenau) Jum. & H. Perrier	22	MADAGASCAR: between Beloha and Ampanihy, 5 km from Beloha	*Liede 2831*
# *C. mevei* Liede	22	MADAGASCAR: Tulear, S de la Table	*Teissier 214* [MSUN]
C. monteridensis Spreng.	22	ARGENTINA: Chaco, Isla del Cerrito	*Liede & Conrad 3019a* [ULM]
C. nodosum (Jum. & H. Perrier) Descoings	22	MADAGASCAR: 15 km from Antanimora to Ambovombé	*Liede 2789*
C. nummulariifolium Hook & Arn.	22	ARGENTINA: Las Lenas, Valle de las Moches	*Liede 3050* [ULM]
C. obovatum (Decne.) Choux	22	MADAGASCAR: ca. 7 km from Ambalamanakana (to Ambosita)	*Liede 2866* [MSUN]
# *C. obtusifolium* L. f.	22	R.S.A.: Eastern Cape Prov., Misfor	*Liede 2925* [UBT]
C. ovalifolium Wight	22	AUSTRALIA: ca. 1.5 km upstream Brown crossing, rd. to Lockhart River Misn.	*Forster 4090*

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
C. papillatum Choux	22	MADAGASCAR: Andringitra	*Liede & Conrad 2862* [MSUN]
C. perrieri Choux	22	MADAGASCAR: s. loc.	*Descoings 28283*
# *C. petignatii* Liede & Rauh	22	MADAGASCAR: Tuléar	*Teissier 269* [UBT]
C. phillipsonianum Liede & Meve	22	MADAGASCAR: Ankarana, Tsingy	*Mangelsdorff M 25* [MSUN]
C. rauhianum Descoings	22	MADAGASCAR: Isalo	*Röösli s.n.*
C. repandum Decne.	22	MADAGASCAR: Ankaratra	*Liede 2867* [MSUN]
C. rossii Rauh	22	MADAGASCAR: Cap St. Marie	*Singer O72478* [MSUN, ZSS]
C. serpyllifolium Kunth	22	ECUADOR: Carachi, El Angel-Tulcan road at Pico Aquer	*Bremer et al. 3372* [MSUN, UPS]
C. sessiliflorum (Decne.) Liede	22	MADAGASCAR: 15 km from Windsor Castle	*Mangelsdorff 13* [MSUN]
***Folotsia* Costantin & Bois**			
F. grandiflora Jum. & H. Perrier	22	MADAGASCAR: Ambovombé to Antanimora, km 24 on sidetrack to Angavo	*Liede 2796* [ULM]
***Funastrum* E. Fourn.**			
F. angustissimum (Anderss.) E. Fourn.	20	ECUADOR: Galapagos Islands, s. loc.	*Stevens s.n.*
F. arenarium (Decne. ex Benth.) Liede	20	MEXICO: San Carlos, road to Beach (Baja California)	*Liede & Conrad 2951* [MSUN, ULM]
F. bilobum (Hook. & Arn.) Macbride	20	MEXICO: Chiapas, La Trinitaria	*Stevens 25744*
F. clausum (Jacq.) Schultes	20	U.S.A.: Florida, Everglades, Reclamation site John Strekl Park	*Liede 3000*
F. crispum (Benth.) Schltr.	11	U.S.A.: Texas, Hueco Tanks State Park	*Liede & Meve 2504* [MO]
# *F. cynanchoides* (Decne.) Schltr. subsp. *cynanchoides*	20	MEXICO: Todos Santos, Sierra Laguna trail (Baja California)	*Liede & Conrad 2958*
F. odoratum (Hemsl.) Schltr.	20	GUATEMALA: Guatemala City, Mixto, Fritz Richter's House	*Liede 3244* [UBT]
***Grisebachiella* Lorentz**			
G. hieronymi Lorentz	20	ARGENTINA: 3 km to Las Moches from Las Lenas (Mendoza)	*Liede & Conrad 3053* [MSUN, ULM]
***Karimbolea* Desc.**			
# *K. macrantha* (Jum. & H. Perrier) Liede & Meve	22	MADAGASCAR: Cap Ste. Marie, 150–200 m from lighthouse along road	*Liede 2829*
***Metaplexis* R. Br.**			
# *M. japonica* (Thunb.) Makino	22, 24	RUSSIA: Primorsk Region	*ex hort. Tartu* [UBT, ULM]
***Metastelma* R. Br.**			
M. parviflorum R. Br.	22	VENEZUELA: Tocopéro	*Liede & Meve 3329* [ULM]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Orthosia* Decne.**			
O. rubens (L. Wms.) Liede ined.	20	MEXICO: Los Azufres, ± 15 km NW of Ciudad Hidalgo	*Conrad 9396* [MSUN, UBT]
O. sp.	20	ECUADOR: Quito	*Liede & Meve 3473* [UBT]
***Oxystelma* R. Br.**			
O. bornouense R. Br.	22	KENYA: Coast, ex hort.	*Liede & Newton 3202* [ULM]
O. esculentum R. Br.	22	EGYPT: Elephantine Island	*Shirley s.n.*
***Pentacyphus* Schltr.**			
P. lehmannii (Schltr.) Liede	22	ECUADOR: Loja, Yananga	*Liede 3333* [UBT]
***Philibertia* Knuth**			
* *P. gilliesii* Lillo	18	ARGENTINA: Mendoza, La Crucecita, ca. 1.5 km from intersection	*Liede 3054* [MSUN]
P. gilliesii Lillo	20	ARGENTINA: Jujuy, Laguna de Yala	*Liede 3111*
P. lysimachioides (Wedd.) T. Meyer	20	BOLIVIA: La Paz, Copacabana peninsula, 8 km from San Pedro	*Liede 3139* [MSUN]
***Platykeleba* N. E. Br.**			
P. insignis N. E. Br.	22	MADAGASCAR: Antanarivo	*Rauh 68500*
***Sarcostemma* R. Br.**			
# *S. acidum* (Roxb.) Voigt	22	NEPAL: NE Dharapani, near Bheri River	*Bruyns s.n.*
# *S. antsiranense* Meve & Liede	22	MADAGASCAR: 75 km N Sambava	*Mangelsdorff 47* [UBT]
# *S. arabicum* Bruyns & P. I. Forst.	22	YEMEN: SW Sana'a	*Radcliffe-Smith 4624* [K]
S. brevipedicellatum P. I. Forst.	22	AUSTRALIA: Gregory North Distr. (Qld.), 86 km from Winton on Boulia Road	*Forster 5876*
* *S. forskaolianum* Schult.	22	YEMEN: 78 km S Dhamar, near branching off Zulmah on Sumarah-Ibb Road	*Voltee 864*
# *S. pearsonii* N. E. Br.	22	R.S.A.: Northern Cape Prov., Smorenskadu	*Liede 2523* [MSUN]
S. resiliens B. R. Adams & R. W. K. Holland	22	ZIMBABWE: 15 km NNW of Rusape	*Albers, Liede & Meve 515* [MSUN]
# *S. stoloniferum* Adams & Holland	44	KENYA: Rumuruti, Kisima Ranch	*Liede & Newton 3174* [MSUN]
S. viminale (L.) R. Br. subsp. *australe* (R. Br.) P. I. Forst.	22	AUSTRALIA: Telowie Gorge (SA)	*Meve & Hellbrügge 723* [MSUN]
S. viminale subsp. *brunonianum* (Wight & Arn.) P. I. Forst.	22	AUSTRALIA: S Kennedy Distr. (Qld.), Thomas Is., NW shore	*Bolton 700* [MSUN]
S. viminale subsp. *brunonianum* (Wight & Arn.) P. I. Forst.	22	YEMEN: Suq As Sabt, Jabal Bura	Wood *3431* [K]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
# *S. viminale* subsp. *odontolepis* (Balf. f.) Meve & Liede	22	KENYA: Kilifi, Arabuko, Sokoke forest	*Field & Powys 186* [MSUN]
* *S. viminale* subsp. *orangeanum* Meve & Liede	22	NAMIBIA: Steinhausen, S Gobabis	*Meve & Liede 599* [MSUN]
* *S. viminale* subsp. *thunbergii* (Don) Liede & Meve	44	R.S.A.: Western Cape Prov., Atties, 1.5 km S Wiedow's River	*Liede & Hammer 2507*
# *S. viminale* subsp. *stipitaceum* (Forssk.) Meve & Liede	22	YEMEN: 1 km E Al Barh	*Noltee 233* [MSUN]
# *S. viminale* (L.) R. Br. subsp. *viminale*	22	MADAGASCAR: between Ampanihy and Androka, 73 km from Ampanihy	*Liede 2701*
***Schizostephanus* Hochst. ex Benth. & Hook. f.**			
S. alatus Hochst. ex K. Schum.	22	KENYA: Kilifi, Chasimba Hills	*Kenealy 9478*
Subtribe: Oxypetalinae K. Schum.			
***Amblystigma* Benth.**			
A. cionophorum Fourn.	22	ARGENTINA: Yerba Buena (Tucumán)	*Liede 3069*
***Araujia* Brot.**			
A. angustifolia Steud.	20	ARGENTINA: Hurlingham, English Club	*Liede 3014* [UBT, ULM]
A. hortorum E. Fourn.	22	ARGENTINA: Buenos Aires, Avenida San Martin	*Liede 3010*
T. brunonis Hook. & Arn.	10	ARGENTINA: Mendoza, Godoy Cruz	*Liede 3058* [ULM]
***Morrenia* Lindl.**			
M. odorata Lindl.	22	ARGENTINA: Chaco, Resistencia	*Liede 3020* [ULM]
***Oxypetalum* R. Br.**			
O. balansae Malme	18	ARGENTINA: Mendoza, Campground "El Challoa"	*Liede & Conrad 3059* [MSUN]
O. coeruleum (D. Don) Decne.	28	ARGENTINA: s. loc.	*ex hort.* [MSUN]
O. ostenii Malme	22	ARGENTINA: Iguacu, Sendero Macuru	*Liede 3042* [MSUN]
O. solanoides Hook. & Arn.	18	ARGENTINA: Buenos Aires, I.N.T.A. area	*Liede & Conrad 3011* [ULM]
***Tweedia* Hook. & Arn.**			
T. brunonis Hook. & Arn.	40	ARGENTINA: Mendoza, Godoy Cruz la Ripiera	*Liede & Conrad 3058* [UBT, ULM]
Subtribe: Tylophorinae (K. Schum.) Liede			
***Blyttia* Arn.**			
B. fruticulosa (Decne.) D. V. Field	22	KENYA: A.I.C. Chemolingot Bore Hole	*Liede 2946* [MSUN]
***Diplostigma* K. Schum.**			
D. canescens K. Schum.	22	TANZANIA: S Pare Mts., NE Mkomazi	*Liede & Meve 3388* [UBT]

Appendix 1. Continued.

Taxon	2*n*	Locality	Voucher
***Pentatropis* R. Br. ex Wight & Arn.**			
P. madagascariensis Decne.	22	MADAGASCAR: 4 km from Tsiomb. between Beloha and Tsihomb	*Liede 2749* [P, UBT]
P. nivalis (J. F. Gmel.) D. V. Field & J. F. Wood	22	KENYA: near Hell's Kitchen	*Meve 949* [MSUN, UBT]
***Pleurostelma* K. Schum.**			
P. cernuum (Decne.) Bullock	22	TANZANIA: W Tanga	*Liede & Meve 3377* [UBT]
***Tylophora* R. Br.**			
T. apiculata K. Schum.	22	KENYA: Tana River Distr. Hewani Forest	*Robertson & Guffa 6894* [MSUN]
T. barbata R. Br.	22	AUSTRALIA: 2 km S Kioloa (NSW)	*Meve & Hellbrügge 790* [MSUN]
T. coriacea Marais	44	MAURITIUS: Isle Rinde	*Bernadi s.n.* [BR, UBT]
T. flexuosa R. Br.	22	PHILIPPINES: Laguna/Quezon, ascent to Mt. Banahaw	*Schneidt & Liede 96-31* [K, ULM]
T. heterophylla N. E. Br.	22	KENYA: Naivasha, Aberdares, 5 km before junction	*Liede 3155* [MSUN, ULM]
T. hirsuta (Burm. f.) Merr.	22	INDIA: Allahabad	*Chaturvedi s.n.* [MSUN]
# *T. indica* (M. Burm.) Merr.	22	INDIA: s. loc.	*Bruyns s.n.* [MSUN]
T. oblonga N. E. Br.	22	CAMEROON: Mt. Cameroon, NW Likombe	*Meve 915* [B, MSUN]
T. perrottetiana Decne.	22	PHILIPPINES: Los Banos, IRRI-Ground	*Liede 3252* [MSUN]
T. tenuipedunculata K. Schum.	22	KENYA: Arabuko Sokoke Forest	*Liede 3200* [MSUN]
T. villosa Blume	22	PHILIPPINES: Luzon, Alaminos	*Schneidt & Liede 96-109* [AUH]
***Vincetoxicum* Wolf**			
# *V. hirundinaria* Medik. ssp. *hirundinaria*	22	F.R.G.: Thüringen, Kyffhäuser-Mountains	*Albers 3646* [MSUN]

SUBTRIBE ASTEPHANINAE (APOCYNACEAE–ASCLEPIADOIDEAE) RECONSIDERED: NEW EVIDENCE BASED ON cpDNA SPACERS[1]

Sigrid Liede[2]

Abstract

The circumscription of the subtribe Astephaninae has a complex and convoluted history due in part to a scarcity of readily observed distinctive morphological characters. In this study the subtribe Astephaninae, as hitherto defined by morphological characters, is reexamined using sequences of the *trn*T-L and *trn*L-F spacer, as well as the *trn*L intron for 12 of its 15 genera. *Eustegia* is found to occupy an isolated basal position in the tribe Asclepiadeae, and *Schizostephanus* is transferred to the Metastelminae. The remaining 10 genera fall into two only distantly related clades of 3 and 7 genera, respectively. The Astephaninae s. str. comprise only 3 South African genera, *Astephanus* s. str., *Microloma*, and *Oncinema*. The other 7 genera form a well-supported clade, the subtribe Tylophorinae. Both groups comprise only Old World genera. All New World taxa studied, even those that have never been formally excluded from *Astephanus*, are found in a distinct, well-supported New World Metastelminae clade. Based on the results presented here one of the key characters of the asclepiads—the corona—has been lost independently at least twice: once in the south African Astephaninae, and once in the New World Metastelminae. This once again underscores the extreme lability of this morphologically complex organ, as well as the difficulties in assessing homology in the asclepiads. A corollary classification of the Asclepiadeae is provided as an Appendix.

Key words: Apocynaceae–Asclepiadoideae, Astephaninae, *trn*L intron, *trn*T-L and *trn*L-F spacer.

The tribe Asclepiadeae, morphologically characterized by pendent pollinia and the possession of true styles (Swarupanandan et al., 1996), forms a monophyletic group occupying an advanced position in the Apocynaceae s.l. according to recent molecular studies (Sennblad, 1997; Civeyrel et al., 1998). The relationships among the ca. 100 Asclepiadeae genera, however, are much less clear. Schumann (1895) distinguished five subtribes using exclusively corona characters. In an attempt to define subtribes on a broader range of apomorphic characters Liede (1997) also recognized five groups, but with a largely different composition. As a sixth subtribe, she added the Gonolobinae following Swarupanandan et al. (1996).

The subtribe Astephaninae Endl. ex Meisn. (1840) originally contained only three genera: *Astephanus* R. Br., *Haemax* E. Mey. (a synonym of *Astephanus*), and *Hybanthera* Endl. (a synonym of *Tylophora* R. Br.). Schumann (1895) considered the subtribe to comprise nine genera: *Microloma* R. Br. from southern Africa, *Adelostemma* Hook. f., and *Henrya* Hemsl. (another synonym of *Tylophora*) from Asia, as well as *Amblystigma* Benth., *Esmeraldia* E. Fourn. (a synonym of *Metastelma* R. Br.), *Hemipogon* Decne., *Mitostigma* Decne., and *Nautonia* Decne. all from South America. *Astephanus*, founded by Brown (1810) exclusively on southern African material and regarded by him as a close relative of *Microloma* (Brown, 1810), was soon extended to contain American taxa without a corona. However, since the absence of a feature—in this case the corona—is not meaningful as an indication of relationship, both the circumscription of the subtribe Astephaninae and of the genus *Astephanus* were in need of reexamination.

Most experts now agree that *Astephanus* s. str. is restricted to the Old World and comprises only three species (*A. marginatus* Schltr., *A. neglectus* Schltr., and *A. triflorus* R. Br.) as stated in Liede (1994). Most of the New World members formerly included in the genus *Astephanus* have been interpreted meanwhile as species with a reduced corona within various currently recognized genera with

[1] Thanks are due to Mark Chase, Kew, for three DNA samples, and to Ashley Nicholas, Pietermaritzburg, and Josefine Schneidt, Edinburgh, for two leaf samples each. The Missouri Botanical Garden Herbarium granted permission for the use of three specimens for this study. Angelika Täuber, Bayreuth, skillfully conducted the laboratory research, and Ulrich Meve, Bayreuth, supported the research during all stages of progress.

[2] Department of Botany, University of Bayreuth, 95444 Bayreuth, Germany. sigrid.liede@uni-bayreuth.de.

coronate flowers and have been transferred accordingly; but there are still some species (e.g., *Astephanus geminiflorus* Deene., *A. multiflorus* T. Mey.) that have not been transferred yet because generic limits in New World Asclepiadeae are still very poorly understood.

Liede (1994) noticed that *Astephanus* s. str. and the closely related *Microloma*, both of which lack a corona, have colorless latex and long, non-verrucose hairs on the adaxial corolla surface, and that they shared this combination of the two features with nine other, coronate genera: *Blyttia* Arn., *Diplostigma* K. Schum., *Goydera* Liede, *Oncinema* Arn., *Pentatropis* R. Br., *Pleurostelma* Baill., *Rhyncharrhena* F. Muell., *Schistostephanus* Hochst. ex Benth., and *Tylophoropsis* N. E. Br. Since these two features are not found in combination elsewhere in Asclepiadeae, she concluded that they most likely represent an apomorphy for this group, and therefore added these nine genera to the Astephaninae (Liede, 1994).

In her synopsis of Asclepiadeae as a whole, Liede (1997) added five more genera to the Astephaninae (*Emicocarpus* K. Schum., *Eustegia* R. Br., *Seshagiria* Ansari & Hemadri, *Tylophora*, and *Vincetoxicum* Medik.), based on the presence of one or both of the following features: long, slender hairs on the adaxial surface of the corolla and sparse clear latex. Traditionally, *Tylophora* has not even been considered to be a member of Asclepiadeae because Schumann (1895) misinterpreted the position of the pollinia in the anther sacs. Schumann's (1895) Tylophoreae K. Schum., except for *Tylophora*, the nomenclatural type, comprise exclusively taxa today considered to be members of Marsdenieae and Stapelieae. Swarupanandan et al. (1996) discussed the position of pollinia in detail, coming to the conclusion that *Tylophora* is a member of Asclepiadeae. Additionally he found (Swarupanandan, 1996) that *Tylophora* also possesses the elongated style typical for Asclepiadeae. Recent molecular studies (Sennblad, 1997; Civeyrel et al., 1998) confirmed its position in Asclepiadeae. Following these results, Liede (1997) put *Tylophoropsis* N. E. Br. into synonymy under *Tylophora*, since it only differs by its pendent pollinia (in contrast to the horizontal ones in *Tylophora*), which does not constitute a fundamental difference.

Only two of the nine genera comprising Schumann's (1895) Astephaninae (*Astephanus* and *Microloma*) are currently recognized in this subtribe. Of the remaining seven genera, *Henrya* has been put into synonymy under *Tylophora*, and *Esmeraldia* into synonymy under *Metastelma*, and the remainder have been transferred as good genera to other Asclepiadeae subtribes by Liede (1997). *Amblystigma* and *Mitostigma* were transferred to the Oxypetalinae since both possess the combination of a corolla tube at least half as long as the total corolla length and a very conspicuous long stylar head, which are apomorphies of Oxypetalinae. The monotypic *Nautonia* agrees in habit, floral structure, and fruit and seed morphology with both *Metastelma*, type genus of Metastelminae, and the closely related *Ditassa*, which differs from *Metastelma* only in that most of its species have a double instead of a simple staminal corona. Even though some species of *Hemipogon* look different in habit and corolla shape, the genus is linked to the Metastelminae by species such as *H. luteus* E. Fourn., and all species agree with Metastelminae very well in fruit and seed morphology (Liede et al., unpublished data). Lastly, the monotypic Asian *Adelostemma*, which was originally described under *Cynanchum* L., and was only excluded from *Cynanchum* because its lack of a corona, agrees with *Cynanchum* s. str. in all other features. Thus *Nautonia*, *Hemipogon*, and *Adelostemma* were all transferred to the Metastelminae.

Liede (1994) conducted a cladistic analysis of the Astephaninae (without the five genera added in Liede, 1997) based on 22 morphological characters. However, this analysis was flawed because *Tylophora*, then still considered a Marsdenieae, was used as the outgroup; while later research showed that it actually belongs to the Asclepiadeae (Swarupanandan et al., 1996) and is congeneric with *Tylophoropsis*, which had been recognized as member of the Astephaninae by Liede (1994), so that *Tylophora* should have been considered a member of the ingroup (Liede, 1997).

Liede's (1994, 1997) concept of Astephaninae was criticized by Bruyns (1999a), who examined a living plant of *Seshagiria* and found it to contain white latex, whereas Liede (1997) had no information on this character. Bruyns (1999b) also conducted a cladistic study on almost the same range of genera, but excluded *Seshagiria*, though he did not exclude it formally from the subtribe, and found *Eustegia* and *Emicocarpus* so closely related that he treated them as one unit in his cladistic analysis. He (Bruyns, 1999b) suggested numerous embellishments to the matrix of morphological characters given in Liede (1994). However, these characters are not necessarily any more useful for cladistic analysis. For example, character 9 (corolline corona present or absent) in Bruyns (1999b) is irrelevant because a corolline corona is absent throughout the ingroup. Bruyns's (1999b) choice of the distantly related genus *Secamone* R. Br. as an outgroup

might contribute to the isolated basal position of *Tylophora* in his strict consensus tree (Bruyns, 1999b). In contrast, Liede (1996) found support for a close relationship between *Tylophora* and *Vincetoxicum* in the occurrence of alkaloids and of 14, 15-seco-pregnanes in both genera, but nowhere else in Asclepiadeae, as far as known. This close relationship has been confirmed both by *mat*K sequence data (Civeyrel et al., 1998) and *rbc*L sequence data (Sennblad, 1997).

The present paper investigates the circumscription of the Astephaninae sensu Liede (1994, 1997) by a molecular marker, the *trn*T-L spacer, *trn*L intron, and *trn*L-F spacer.

Materials and Methods

Taxa

Material was available of all genera of Astephaninae sensu Liede (1994, 1997) except three (*Emicocarpus, Rhyncharrhena, Seshagiria;* Table 1). *Gymnema* R. Br. and *Cionura* Griseb., members of the Marsdenieae, and *Ceropegia* L. and *Stapelia* L., members of the Ceropegieae, were chosen as outgroups belonging to different tribes. In the Asclepiadeae, a wide range of different genera was included, focusing on genera and species without a corona. They are listed in Table 1 (refer here for authors of species) according to their classification by Liede (1997).

DNA extraction and PCR

DNA was isolated from fresh or dried leaf tissue according to Doyle and Doyle (1987). PCR primers and protocol for the plastid *trn*T-*trn*L and *trn*L-*trn*F spacers as well as the *trn*L intron follow Taberlet et al. (1991). Sequences were obtained on an ABI Prism Model 310 Version 3.0 sequencer. Of the 43 taxa, 36 have been sequenced for this study; the remaining seven sequences had been deposited at EMBL in the course of earlier studies of the author (for accession numbers, see Table 1).

Data analysis

Sequences were pre-aligned with Perkin Elmer Sequence Navigator Version 1.0.1; the alignment was cleaned manually. The sequence alignment (available from the author) comprises 43 taxa and 2088 characters (1076 sequence characters and 18 indels in the *trn*T-*trn*L intron (primers a and b), 547 sequence characters and 9 indels between the two *trn*L-exons (primers c and d), and 432 sequence characters and 6 indels in the *trn*L-*trn*F intron (primers e and f)); 45 data cells are unknown and were coded as missing characters.

Phylogenetic analysis and tests for clade support were performed using PAUP version 4.0d65 (PPC; Swofford, 1998), on a Macintosh Powerbook G3. Indels were coded as "missing" characters throughout; possibly parsimony-informative indels were coded separately following the "simple gap coding" method of Simmons and Ochoterena (2000). In two areas with very irregular and potentially ambiguous indel pattern in the *trn*L-F spacer (bp 340–408, 693–758), no separate indel coding was performed. Different lengths of poly-chains of more than 5 bp have not been coded as indels either because the length of these chains has been found to be variable even within the same species (Liede, unpublished data).

For parsimony analysis, first all sequence characters were analyzed. Then the 33 separately coded indels were added. Heuristic search for both data sets was conducted in two steps: first, starting trees were obtained setting addition sequence at "random" and 1000 replicates, "MulTrees" and "Steepest descent" off. Then, these starting trees were subjected to TBR branch swapping, "MulTrees" on, "Steepest descent" off.

Bootstrap search (1000 replicates) was conducted under the "fast" stepwise addition type of search. Jackknife resampling (1000 replicates) was set to 50% deletion, and "Jac" resampling; the other settings were identical to the bootstrap settings.

Results

Parsimony analysis of all sequence characters (yielding 202 parsimony-informative characters) results in 104 most parsimonious trees (l = 611, CI = 0.8491, RI = 0.8777, RC = 0.7455). Adding the indels yields 234 parsimony informative characters (indel 8 of the *trn*T-L spacer is not parsimony informative), and analysis results in 40 most parsimonious trees (l = 670, CI = 0.8239, RI = 0.8671, RC = 0.7144). The strict consensus tree resulting from both analyses is shown in Figure 1.

In both analyses, the ingroup splits into two major, well-supported clades, the *Astephanus*-clade and the *Tylophora*-clade, though the position of taxa within the *Tylophora*-clade is less well established. The topology of the strict consensus resulting from the addition of the indels changes only the position of the *Pentatropis*-clade from the base of the *Tylophora*-clade to an unresolved subclade of the *Tylophora*-clade (dashed line in Fig. 1) and distinguishes two unsupported subclades of the

Table 1. Voucher and locality information for plant material used in this study. All taxa belong to the Apocynaceae–Asclepiadoideae. * indicates species without a corona.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
OUTGROUPS			
Marsdenieae			
Cionura erecta (R. Br.) Griseb.	Turkey	*Heyne 120* (UBT)	AJ410172 AJ410173 AJ410174
Gymnema sylvestre (Retz.) Schult.	Cameroon	*Meve 919* (UBT)	AJ402118 AJ402137 AJ402142
Ceropegieae			
Ceropegia nilotica Kotschy	Kenya	*Masinde 836* (MSUN)	AJ402117 AJ402138 AJ402141
Stapelia glanduliflora Masson	South Africa	*Albers & Meve 04* (MSUN)	AJ402127 AJ402128 AJ402151
Asclepiadeae–Asclepiadinae			
Asclepias syriaca L.		ex hort. Münster; in cult. Münster	AJ410178 AJ410179 AJ410180
Gomphocarpus physocarpus E. Mey.	South Africa	*Nicholas 2829* (UDW)	AJ290877 AJ290876 AJ290875
Pergularia daemia (Forssk.) Chiov.	Tanzania	*Masinde 888* (UBT)	AJ290891 AJ290892 AJ290893
Asclepiadeae–Metastelminae (Old World)			
Biondia henryi (Warb. ex Schltr. & Diels) Tsiang & P. T. Li	China	*Deng 90203* (MO)	AJ410190 AJ410191 AJ410192
Cynanchum auriculatum Royle ex Wight	China	ex hort. Nanking (UBT)	AJ410196 AJ410197

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
			AJ410198
Cynanchum ellipticum (Harv.) R. A. Dyer	South Africa	*Liede 2933* (UBT)	AJ290847
			AJ290846
			AJ290845
Pentarrhinum insipidum E. Mey.	South Africa	*Liede 2940* (UBT)	AJ410232
			AJ410233
			AJ410234
Asclepiadeae–Metastelminae (New World)			
*"*Astephanus" geminiflorus* Decne.	Chile	*Heyne 103* (MSUN)	AJ410181
			AJ410182
			AJ410183
*Ditassa grazielae (Fontella & Marquete) Rapini ined.	Brazil	*Omlor 147* (MJG)	AJ410202
			AJ410203
			AJ410204
**Grisebachiella hieronymi* Lorentz	Argentina	*Liede & Conrad 3052* (MSUN, ULM)	AJ410211
			AJ410212
			AJ410213
Metastelma schaffneri A. Gray	Mexico	*Liede & Conrad 2962* (UBT)	AJ410214
			AJ410215
			AJ410216
**Nautonia nummularia* Decne.	Argentina	*Liede & Conrad 3031* (ULM)	AJ410226
			AJ410227
			AJ410228
Asclepiadeae–Oxypetalinae			
**Melinia candolleana* (Hook. & Arn.) Decne.	Argentina	*Liede & Conrad 3055* (ULM)	AJ410175
			AJ410176
			AJ410177
**Melinia parviflora* (Malme) A. Krapovickas & S. Cáceres Moral	Argentina	*Liede & Conrad 3113* (UBT)	AJ410223
			AJ410224
			AJ410225
Schistogyne sylvestris Hook. & Arn.	Argentina	*Liede & Conrad 3024* (K, MO, MSUN, ULM)	AJ410244
			AJ410245
			AJ410246

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
INGROUP (Asclepiadeae–Astephaninae sensu Liede, 1994, 1997)			
**Astephanus neglectus* Schltr.	South Africa	*Goldblatt 2042* (MO)	AJ410184 AJ410185 AJ410186
**Astephanus triflorus* R. Br.	South Africa	*Williams 659 (MO)*	AJ410187 AJ410188 AJ410189
Blyttia fruticulosum (Decne.) D. V. Field	Kenya	*Liede & Newton 2946* (UBT)	AJ410193 AJ410194 AJ410195
Diplostigma canescens K. Schum.	Kenya	*Liede & Newton 3214* (UBT)	AJ410199 AJ410200 AJ410201
Eustegia minuta (L.f.) N. E. Br.	South Africa	*Bruyns 4357* (K; MWC 3291)	AJ410205 AJ410206 AJ410207
Goydera somaliense Liede	Somalia	*Thulin & Bashir 6882* (UPS)	AJ410208 AJ410209 AJ410210
**Microloma sagittatum* R. Br.	South Africa	*Mere & Liede 616* (MSUN)	AJ410217 AJ410218 AJ410219
**Microloma tenuifolium* K. Schum.	South Africa	*Albers s.n.* (MSUN)	AJ410220 AJ410221 AJ410222
Oncinema lineare (L.f.) Bullock	South Africa	*Bruyns s.n.* (K; MWC 3290)	AJ410229 AJ410230 AJ410231
Pentatropis madagascariensis Decne.	Madagascar	*Liede 2749* (UBT)	AJ410235 AJ410236 AJ410237
Pentatropis nivalis (J. F. Gmel.) D. V. Field & J. R. I .Wood	Kenya	*Mere 949* (UBT)	AJ410238 AJ410239

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No. *trn*T-L spacer *trn*L intron *trn*L-F spacer
			AJ410240
Pleurostelma cernuum (Decne.) Bullock	Tanzania	*Liede & Meve 3377* (UBT)	AJ410241
			AJ410242
			AJ410243
Schizostephanus alatus Hochst. ex K. Schum.	Kenya	*Noltee s.n. sub IPPS 8111* (UBT)	AJ410247
			AJ410248
			AJ410249
Tylophora anomala N. E. Br.	Cameroon	*Meve 916* (K, UBT)	AJ410250
			AJ410251
			AJ410252
Tylophora apiculata K. Schum.	Kenya	*Robertson 7016* (UBT)	AJ410253
			AJ410254
			AJ410255
Tylophora flanaganii Schltr.	South Africa	*Nicholas 2839* (UDW)	AJ410256
			AJ410257
			AJ410258
Tylophora flexuosa R. Br. var. perrottetiana (Decne.) Schneidt ined.	Philippines	*Liede 3252* (UBT)	AJ290915
			AJ290916
			AJ290917
Tylophora heterophylla A. Rich.	Kenya	*Liede & Newton 3155* (UBT)	AJ410259
			AJ410260
			AJ410261
Tylophora indica (Burm. f.) Merrill	India	*Bruyns s.n.* (UBT)	AJ410262
			AJ410263
			AJ410264
Tylophora sylvatica Decne.	Africa (ex hort.)	*Valck s.n.* (UBT)	AJ410265
			AJ410266
			AJ410267
Vincetoxicum atratum Morr. & Decne.	China	*Schneidt 96-137* (ABD)	AJ410268
			AJ410269
			AJ410270
Vincetoxicum carnosum Benth.	Borneo	*Schneidt 95-97* (ABD, L)	AJ410271
			AJ410272
			AJ410273

Table 1. Continued.

Species	Origin	Voucher	EMBL Accession No.
			*trn*T-L spacer
			*trn*L intron
			*trn*L-F spacer
Vincetoxicum hirundinaria Medic.	Germany	*Meve s.n.* (UBT)	AJ410274
Vincetoxicum stocksii S. I. Ali & S. Khatoon	Pakistan	*Ali & Khatoon s.n.* (GA)	AJ410275
			AJ410276
			AJ410277
			AJ410278
			AJ410279

Tylophora-clade (dotted lines in Fig. 1). In both analyses, *Eustegia* forms the most basal clade in Asclepiadinae, followed by the *Astephanus*-clade. The New World Metastelminae (including the three representatives of Oxypetalinae as a subclade) follow and are equally well supported. The main clades that follow are the Old World Metastelminae (including *Schizostephanus*), the Asclepiadinae, and, last, the *Tylophora*-clade, which comes out in the most derived position.

Discussion

The genera placed in Astephaninae by Liede (1994, 1997) are not monophyletic according to the results of the cpDNA analyis.

Schizostephanus is more closely related to *Pentarrhinum* and the Old World species of *Cynanchum* than to other Astephaninae genera. *Schizostephanus* shares with *Cynanchum* the highly fused corona of staminal and interstaminal parts as well as the reniform leaf bases and possesses thus the characters listed as synapomorphies for the Metastelminae Endl. ex Meisn. sensu Liede (1997), to which it is consequently transferred.

Eustegia shows no close affinity, either morphologically or molecularly, to any other genus included in the analysis and takes a basal position within the tribe Asclepiadeae. Bruyns (1999b) has examined the close relationship of *Eustegia* and *Emicocarpus*, in particular with reference to their unique 3-seriate corona. The isolated position of *Eustegia* and *Emicocarpus* argued for by Bruyns (1999b) is supported by the present results at least for *Eustegia*. Both genera are monotypic, and both occupy a very restricted distribution area, *Eustegia* in the Western Cape, and *Emicocarpus* around Maputo (Mozambique), suggesting that these two genera might be relics of a once more widespread and diverse group of Asclepiadeae.

The three southern African genera *Astephanus*, *Microloma*, and *Oncinema* form a clade with 100% bootstrap and jackknife support in the present analysis. Bruyns and Linder (1991) listed "similarly shaped, small subcoriaceous leaves and slender climbing habit and the similarly elongated style apex" as well as clear latex as common characters of these three genera. While none of these characters alone is unique in Asclepiadeae, the combination of all three can be used to characterize the Astephaninae-clade. All species of *Microloma* investigated possess a chromosome number of $x = 10$ (Albers et al., 1993), while the vast majority of Asclepiadoideae, 96 of the 104 genera studied, possess $x = 11$ (Albers & Meve, 2001 this volume).

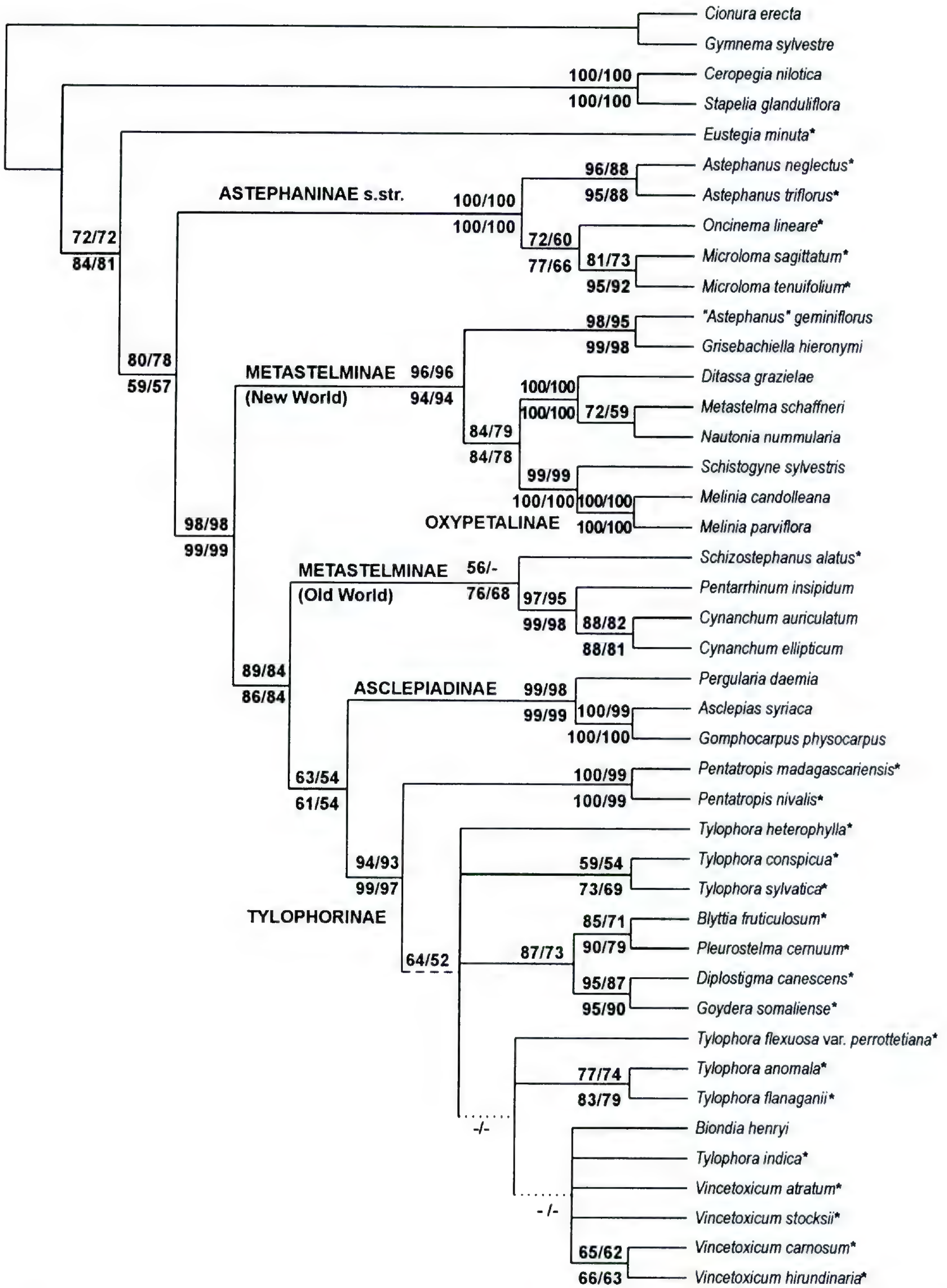


Figure 1. Strict consensus tree of the 104 most parsimonious trees (l = 611, CI = 0.8494, RI = 0.8777, RC = 0.7455) resulting from parsimony analysis of all sequence characters and of the 40 most parsimonious trees (l = 670, CI = 0.8239, RI = 0.8671, RC = 0.7144) resulting from analysis of all sequence characters and all indels. Asterisks denote taxa included in Astephaninae sensu Liede (1997). Dotted lines indicate clades not retrieved in the analysis without the indels; the dashed line indicates a clade not retrieved in the analysis including the indels. Numbers indicate bootstrap/jackknife values and refer to the analysis without the indels above branches and with the indels below the branches.

Unfortunately, there are no cytological data available on this potential synapomorphy for *Astephanus* and *Oncinema*. With *Astephanus*, this clade includes the nomenclatural type of the subtribe Astephaninae. The Astephaninae s. str. therefore include only three genera: *Astephanus*, *Microloma*, and *Oncinema*. This subtribe is restricted to the Old World, only occurring in southern Africa; the results of the present analysis confirm that none of the corona-less New World taxa is a member of *Astephanus* R. Br. Records of *Microloma incanum* Decne. in Madagascar (Meve & Liede, 1995) might point to a once more widespread distribution of Astephaninae s. str. in Africa, but the material is too scanty to postulate such an extended distribution area with certainty. The Astephaninae s. str. occupy the second most basal position in the Asclepiadeae (Fig. 1). As both most basal clades (*Eustegia* and Astephaninae s. str.) occur in southern Africa, it can be speculated that the origin of Asclepiadeae probably lies in the southern African area.

New World Metastelminae (sensu Liede, 1997) (including Oxypetalinae), the Asclepiadinae, and the Old World Metastelminae form monophyletic clades between Astephaninae s. str. and a clade comprising the remainder of the genera attributed to Astephaninae sensu Liede (1994, 1997) together with *Biondia*, which was formerly considered a member of Metastelminae. The split of Metastelminae sensu Liede (1997) into an Old World and a New World clade has been observed in an analysis of the genera *Sarcostemma* s.l. (Liede & Täuber, 2000) and *Cynanchum* (Liede & Täuber, in prep.), and is discussed in depth in the latter paper. The close relationships between Oxypetalinae and the New World clade of Metastelminae is at present under study (Liede & Goyder, unpublished results).

Biondia, *Blyttia*, *Diplostigma*, *Goydera*, *Pentatropis*, *Pleurostelma*, *Tylophora*, and *Vincetoxicum* form a well-supported clade in the analysis of sequence characters alone, and their close relationship is supported by the indel pattern, as the inclusion of indels raises both bootstrap and jackknife values (Fig. 1). Resolution within this clade is low, with the exception of the well-supported subclade formed by the four small African genera *Blyttia*, *Diplostigma*, *Goydera*, and *Pleurostelma*. The basal position of *Pentatropis* is weakly supported in the analysis of sequence characters alone, and the *Pentatropis*-clade forms an unresolved subclade of the main clade in the analysis including the indels (Fig. 1). As a corollary, *Vincetoxicum carnosum* Benth. always appears in the unresolved *Tylophora*-clade and should not be transferred to *Pentatropis* as was suggested earlier due to its morphology (Liede, 1994). Common morphological characters of the genera in the *Tylophora*-clade include rather small, inconspicuous flowers with a gynostegial corona of five separate staminal parts, and small, often disk-shaped pollinia attached to the corpusculum via cylindrical caudicles. Latex is clear in almost all taxa except for some species of *Tylophora*, which have white or yellowish latex. Non-verrucose hairs on the adaxial corolla surface are also present (Liede, 1994, 1997), mainly at the entrance of the tube. *Rhyncharrhena*, one of the genera for which no sequenceable material could be obtained, also shows these characters. Its only species was originally described under *Pentatropis* (*P. linearis* Decne.), and while the characters listed by Wilson (1980) may warrant its recognition as a distinct genus, its morphology indicates a position between *Pentatropis* and *Tylophora*, in particular with regard to corona and inflorescence structure. Therefore, *Rhyncharrhena* is tentatively placed in the Tylophorinae.

The *Tylophora*-clade has been analyzed for a second marker, ITS (Liede et al., in press), for which the same pattern has been found with a strongly supported clade and weak internal resolution. An attempt to align ITS sequences of the three Astephaninae s. str. genera with those of *Tylophora* and its allies failed (Liede, unpublished data), which is not surprising considering that ITS in general has a much faster rate of change than the cpDNA regions analyzed here. For the members of the *Tylophora*-clade, the name Tylophorinae K. Schum. is appropriate, even though Schumann (1895) used it to circumscribe a set of genera now classified as Marsdenieae and Ceropegieae except for the type genus, *Tylophora* (Liede & Albers, 1994). While it is unfortunate that *Tylophora* is the only genus common to Tylophoreae sensu Schumann (1895) and Tylophorinae as circumscribed here, Article 47 and Recommendation 19A.2 of the ICBN (Greuter et al., 2000) indicate that this is the correct name for the taxon.

The Tylophorinae, with the two species-rich genera *Tylophora* and *Vincetoxicum*, are distributed throughout the Old World, with a center of generic diversity in East Africa. Contrary to traditional views that taxa with a very simple floral structure are primitive in Asclepiadeae, Tylophorinae occupy an advanced position within the Asclepiadeae, which comes out as the crown clade in our study. Sennblad (1997), Civeyrel et al. (1998), Fishbein (2001 this volume), and Potgieter and Albert (2001 this volume) have analyzed a smaller number of Asclepiadeae taxa. In the *rbc*L study of Sennblad, the *Tylophora/Vincetoxicum*-clade comes out as sis-

ter to an Oxypetalinae/Gonolobinae-clade in the most derived position. The strange position of "*Cynanchum*" in this study (Sennblad, 1997) is explained by the choice of a New World representative (*C. serpyllifolium* Kunth) that is not a member of *Cynanchum* s. str. (Liede & Täuber, in prep.). *Schizostephanus* (a member of Old World Metastelminae) and *Asclepias/Calotropis* (Asclepiadinae) are unresolved sisters to the Tylophorinae/Oxypetalinae/Gonolobinae-clade (Sennblad, 1997), for which, unfortunately, no support values are given. In the *mat*K study of Civeyrel et al. (1998) the *Tylophora/Vincetoxicum*-clade again comes out as sister to an Oxypetalinae/Gonolobinae-clade in a more derived position than *Pergularia* (Asclepiadinae) and *Pentarrhinum* (Old World Metastelminae). In the *mat*K study of Fishbein (2001) the *Tylophora/Vincetoxicum*-clade forms one of the unresolved Asclepiadeae-clades. In the combined *trn*L-F spacer, *trn*L intron and morphological fruit character study of Potgieter and Albert (2001), the three unresolved clades *Oxystelma* (one species), Tylophorinae (four species), and Asclepiadinae (seven species) take the most derived position in Asclepiadeae. From these studies it becomes clear that Tylophorinae are one of the most derived groups of Asclepiadeae, so that their rather simple floral structure has to be regarded as an advanced rather than a primitive character.

The fact that Tylophorinae most likely represent an advanced group of genera is underlined by the distribution of *Vincetoxicum*. Of all Asclepiadoideae (and even Apocynaceae), *Vincetoxicum* has radiated furthest to the north (as far as Sweden), away from the sub-tropical African center of origin of the subfamily (Good, 1951). *Vincetoxicum* possesses a remarkable potential to expand range distribution, probably due to its capacity of self-fertilization, unusual in Asclepiadoideae (Lumer & Yost, 1995), and its rapid spread throughout the United States and Canada after its accidental introduction in several places along the east coast of North America in the second half of the last century has been well documented (e.g., Sheeley & Raynal, 1996).

No material for sequencing was available for *Seshagiria*, a recently described Indian genus of doubtful affinity (Ansari & Hemadri, 1971a, 1971b). Its floral structure is strongly reminiscent of that in some members of *Pentatropis* (e.g., *P. oblongifolia* (Cost.) Liede), but its stout, verrucose fruits are otherwise unknown in Tylophorinae. Bruyns (1999a) has pointed out that *Seshagiria* has white latex, but neither he (Bruyns, 1999a) nor Ansari and Hemadri (1971a, 1971b) have any suggestions as to the relationships of this rare monotypic genus. Thus, for the time being, *Seshagiria* is considered as a genus *incertae sedis* in the Asclepiadeae.

Appendix 1 presents a corollary classification of the Asclepiadeae.

Literature Cited

Albers, F. & U. Meve. 2001. A karyological survey of Asclepiadoideae, Periplocoideae, and Secamonoideae, and evolutionary considerations within Apocynaceae s.l. Ann. Missouri Bot. Gard. 88: 624–656.

———, S. Liede & U. Meve. 1993. Deviating chromosome numbers in Asclepiadaceae. Nordic J. Bot. 13: 37–39.

Ansari, M. Y & K. Hemadri. 1971a. *Seshagiria* Ansari et Hemadri—A new genus of Asclepiadaceae from Sahyadri Ranges, India. Indian Forester 97: 126–127.

——— & ———. 1971b. *Seshagiria* Ansari et Hemadri (Asclepiadaceae) from Maharashtra State, India—Additional data. Bull. Bot. Surv. India 13: 357–358.

Brown, R. 1810. On the Asclepiadeae, a natural order of plants separated from the Apocineae of Jussieu. [Preprint of Mem. Wern. Nat. Hist. Soc. 1: 12–78 (1811).]

Bruyns, P. V. 1999a. Subtribes and genera of Asclepiadeae—A response to Liede. Taxon 48: 23–26.

———. 1999b. The systematic position of *Eustegia* R.Br. (Apocynaceae–Asclepiadoideae). Bot. Jahrb. Syst. 121: 19–44.

——— & P. H. Linder. 1991. A revision of *Microloma* R.Br. (Asclepiadaceae). Bot. Jahrb. Syst. 112: 453–527.

Civeyrel, L., A. Le Thomas, K. Ferguson & M. W. Chase. 1998. Critical reexamination of palynological characters used to delimit Asclepiadaceae in comparison to molecular phylogeny obtained from plastid *mat*K sequences. Molec. Phylogenet. Evol. 9: 517–527.

Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. Bot. Soc. Amer. 19: 11–15.

Fishbein, M. 2001. Evolutionary innovation and diversification in the flowers of Asclepiadaceae. Ann. Missouri Bot. Gard. 88: 603–623.

Good, R. 1951. Atlas of the Asclepiadaceae. New Phytol. 51: 198–209.

Greuter, W., J. McNeill, F. R. Barrie, H. M. Burdet, V. Demoulin, T. S. Filgueiras, D. H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland & D. L. Hawksworth (Editors). 2000. International Code of Botanical Nomenclature (Saint Louis Code). Regnum Veg. 138.

Liede, S. 1994. Myth and reality of the subtribe Astephaninae K. Schum. (Asclepiadaceae). Bot. J. Linn. Soc. 114: 81–98.

———. 1996. *Cynanchum–Rhodostegiella–Vincetoxicum– Tylophora*: New considerations on an old problem. Taxon 45: 193–211.

———. 1997. Subtribes and genera of the tribe Asclepiadeae (Apocynaceae–Asclepiadoideae)—A synopsis. Taxon 46: 233–247.

——— & F. Albers. 1994. Tribal disposition of Asclepiadaceae genera. Taxon 43: 201–231.

——— & A. Täuber. 2000. *Sarcostemma* R. Br. (Apocynaceae–Asclepiadoideae)—A controversial generic circumscription reconsidered: Evidence from *trn*L-F spacers. Pl. Syst. Evol. 225: 133–140.

———, ——— & J. Schneidt. (In press). Molecular considerations on the Tylophorinae K. Schum. (Apocynaceae–Asclepiadoideae). Edinburgh J. Bot.

Lumer, C. & S. E. Yost. 1995. The reproductive biology of *Vincetoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York State. Bull. Torrey Bot. Club 122: 12–23.

Meisner, C. F. 1840. Asclepiadaceae. Pp. 266–271 *in* Genera Plantarum Vascularum. Weidmann, Leipzig.

Meve, U. & S. Liede. 1995. *Microloma* in Madagascar. Asklepios 65: 18–20.

Potgieter, K. & V. Albert. 2001. Phylogenetic relationships within Apocynaceae s.l. based on *trn*L intron and *trn*L-F spacer sequences and propagule characters. Ann. Missouri Bot. Gard. 88: 523–549.

Schumann, K. 1895. Asclepiadaceae. Pp. 189–305 *in* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien, vol. 4.1. Engelmann, Leipzig.

Sennblad, B. 1997. Phylogeny of the Apocynaceae s.l. Acta Universitatis Upsaliensis, Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 295.

Sheeley, S. E. & D. J. Raynal. 1996. The distribution and status of species of *Vincetoxicum* in eastern North America. Bull. Torrey Bot. Club 123: 148–156.

Simmons, M. P. & H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49: 369–381.

Swarupanandan, K., J. K. Mangaly, T. K. Sonny, K. Kishorekumar & S. Chand Basha. 1996. The subfamilial and tribal classification of the family Asclepiadaceae. Bot. J. Linn. Soc. 120: 327–369.

Swofford, D. L. 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods), Vers. 4. Sinauer, Sunderland, Massachusetts.

Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Molec. Biol. 17: 1105–1109.

Wilson, K. L. 1980. The genus *Rhyncharrhena* (Asclepiadaceae). Telopea 2: 35–39.

Appendix 1. Corollary taxonomic changes within the Asclepiadeae.

Metastelminae Endl. ex Meisn., Pl. Vasc. Gen. 1: 257, 267; 2: 174. 1840. TYPE: *Metastelma* R. Br.

Metastelma R. Br., Asclepiadeae 41. 1810. TYPE: *Metastelma parviflorum* (Sw.) R. Br. ex Schult.

Schizostephanus Hochst. ex K. Schum., Bot. Jahrb. Syst. 17: 139. 1893. TYPE: *Schizostephanus alatus* Hochst. ex K. Schum.

Astephaninae Endl. ex Meisn., Pl. Vasc. Gen. 1: 257, 266; 2: 174. 1840. TYPE: *Astephanus* R. Br.

Astephanus R. Br., Asclepiadeae 43. 1810. TYPE: *Astephanus triflorus* (L. f.) R. Br. ex Schult., in Roem. & Schult., Syst. Veg. 6: 122. 1820.

Haemax E. Mey., Comm. Pl. Afr. Austr. 223. 1838. TYPE: *Haemax massonii* E. Mey.

Microloma R. Br., Asclepiadeae: 42. 3 Apr. 1810. TYPE: *Microloma sagittatum* (L.) R. Br.

Oncinema Arn., Edinburgh New Philos. J. 17: 261. 1834. TYPE: *Oncinema roxburghii* Arn.

Glossostephanus E. Mey., Comm. Pl. Afr. Austr. 217. 1838. TYPE: *Glossostephanus linearis* (L. f.) E. Mey.

Tylophorinae (K. Schum.) Liede, stat. nov. Basionym: Tylophoreae K. Schum., in Engl. & Prantl, Nat. Pflanzenfam. 4: 209. 1895. TYPE: *Tylophora* R. Br.

Biondia Schltr., Bot. Jahrb. Syst. 36 (Beibl. 82): 91. 1905. TYPE: *Biondia chinensis* Schltr.

Blyttia Arn., in Jardine & Johnston, Mag. Zool. Bot. 2: 420. 1838. TYPE: *Blyttia arabica* Arn. (*Haplostemma* Endl.).

Haplostemma Endl., Gen. Pl. Suppl. 3: 75. 1843, nom. illeg.

Diplostigma K. Schum., in Engler, Pflanzenw. Ost-Afrikas C: 324. 1895. TYPE: *Diplostigma canescens* K. Schum.

Goydera Liede, Novon 3: 265. 1993. TYPE: *Goydera somaliense* Liede.

Pentatropis R. Br. ex Wight & Arn., in Wight, Contr. Bot. India 52. 1834. TYPE: *Pentatropis microphylla* (Roth ex Schult.) Wight & Arn.

Ischnostemma King & Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74: 532. 1908. TYPE: *Ischnostemma selangoricum* King & Gamble.

Pseudopentatropis Costantin, in Lecomte, Fl. Indo-Chine 4: 61. 1912. TYPE: *Pseudopentatropis oblongifolia* Costantin.

Strobopetalum N. E. Br., Bull. Misc. Inform. Kew 1894: 335. 1894. TYPE: *Strobopetalum bentii* N. E. Br.

Pleurostelma Baill., Hist. Pl. 10: 266. 1890. TYPE: *Pleurostelma grevei* Baill.

Microstephanus N. E. Br., Bull. Misc. Inform. Kew 1895: 249. 1895. TYPE: *Microstephanus cernuus* (Decne.) N. E. Br.

Podostelma K. Schum., Bot. Jahrb. Syst. 17: 133. 1893. TYPE: *Podostelma schimperi* (Vatke) K. Schum.

Rhyncharrhena F. Muell., Fragm. 1: 128. 1859. TYPE: *Rhyncharrhena atropurpurea* F. Muell.

Tylophora R. Br., Prodr. 460. 1810. TYPE: *Tylophora flexuosa* R. Br.

Amblyoglossum Turcz., Bull. Soc. Imp. Naturalistes Moscou 25: 310. 1852. TYPE: *Amblyoglossum brevipes* Turcz.

Belostemma Wall. ex Wight, Contr. Bot. India 52. 1834. TYPE: *Belostemma hirsutum* (Wall.) Wall. ex Wight.

Hoyopsis H. Lév., Repert. Spec. Nov. Regni Veg. 13: 262. 1914. TYPE: *Hoyopsis dielsii* H. Lév.

Hybanthera Endl., Prodr. Fl. Norfolk. 59. 1833. TYPE: *Hybanthera biglandulosa* Endl.

Iphisia Wight & Arn., in Wight, Contr. Bot. India: 52. 1834. TYPE: not designated.

Nanostelma Baill., Hist. Pl. 10: 247. 1890. TYPE: *Nanostelma congolanum* Baill.

Neohenrya Hemsl., Bull. Torrey Bot. Club 19: 97. 1892. TYPE: *Neohenrya angustiniana* (Hemsl.) Hemsl. (*Henrya* Hemsl. non *Henrya* Nees ex Benth., *Henryastrum* Happ).

Oncostemma K. Schum., Bot. Jahrb. Syst. 17: 148. 1893. TYPE: *Oncostemma cuspidatum* K. Schum.

Tylophoropsis N. E. Br., Gard. Chron., ser. 3. 16: 244. 1894. TYPE: not designated.

Vincetoxicum Wolf, Gen. Pl.: 130. 1776. TYPE: *Vincetoxicum hirundinaria* Medik.

Alexitoxicon St.-Lag., Ann. Soc. Bot. Lyon 7: 67. 1880, nom. illeg.

Antitoxicum Pobed., Fl. URSS 18: 674. 1952, nom. illeg.

Pentabothra Hook. f., Fl. Brit. Ind. 4: 18. 1883. TYPE: *Pentabothra nana* (Buch.-Ham. ex Wight) Hook. f.

Pycnostelma Bunge ex Decne., in A. de Candolle, Prodr. 8: 512. 1844. TYPE: *Pycnostelma chinensis* Bunge ex Decne.

ELUCIDATING DEEP-LEVEL PHYLOGENETIC RELATIONSHIPS IN SAXIFRAGACEAE USING SEQUENCES FOR SIX CHLOROPLASTIC AND NUCLEAR DNA REGIONS[1]

Douglas E. Soltis,[2] Robert K. Kuzoff,[3] Mark E. Mort,[4] Michael Zanis,[5] Mark Fishbein,[5] Larry Hufford,[5] Jason Koontz,[6] and Mary K. Arroyo[7]

Abstract

To elucidate relationships at deep levels within Saxifragaceae we analyzed phylogenetically a data set of sequences for six DNA regions, four representing the chloroplast genome (*rbc*L, *mat*K, *trn*L-*trn*F, *psb*A-*trn*H) and two from the nuclear genome (ITS and expansion segments of the 26S rDNA). A total of 6676 bp was aligned per taxon, 4559 bp and 1878 bp from the chloroplast and nuclear genomes, respectively. Chloroplast and nuclear trees agreed closely, prompting analysis of a combined, six-gene data set. Application of both parsimony and maximum likelihood methods yielded similar topologies. The use of different ITS alignments and the exclusion of hard-to-align ITS regions had little impact on either the final nuclear-based topology, or the shortest trees from the analysis of six genes. The affinities of two monotypic genera (*Saxifragella* and *Saxifragodes*) endemic to Tierra del Fuego were elucidated. *Saxifragella* is an early branching member of the North Temperate genus *Saxifraga* s. str.; *Saxifragodes* is sister to *Cascadia*, a genus endemic to Oregon and Washington. Long-distance dispersal from east Asia or western North America to South America may have played an important role in forming these and other similar disjunctions in the family. A number of well-supported clades are present, including *Saxifraga* s. str., *Micranthes*, *Saxifragopsis/Astilbe*, *Chrysosplenium/Peltoboykinia*, and the *Boykinia* and *Heuchera* groups. The use of additional characters has provided greatly increased resolution and internal support at deep levels. Saxifragaceae comprise two major lineages: *Saxifraga* s. str. (including *Saxifragella*) and all other genera of the family (the heucheroids). This major split is accompanied by general biogeographical and morphological differences. Whereas *Saxifraga* s. str. is largely arctic to alpine in occurrence, the heucheroid clade is largely temperate in distribution. *Saxifraga* s. str. has a relatively uniform floral morphology (generally actinomorphic; 5 sepals, 5 petals, 10 stamens, 2 carpels), whereas the heucheroid clade encompasses actinomorphic and zygomorphic forms, as well as variation in the number of sepals, petals, stamens, and carpels. Deep-level relationships within both *Saxifraga* s. str. and the heucheroid clade are well resolved and supported. A phylogenetic classification of the family is provided.

Key words: molecular systematics, phylogeny, Saxifragaceae, taxonomy.

Saxifragaceae are a eudicot family of approximately 30 genera of herbaceous perennials, about half of which are monotypic (Table 1). The largest genera include *Heuchera* (about 50 species; Rosendahl et al., 1936), *Chrysosplenium* (57 species; Hara, 1957), a narrowly defined *Saxifraga* (over 300 species; Gornall, 1987; Webb & Gornall, 1989), and the *Micranthes* clade (= *Saxifraga* sect. *Micranthes*; approximately 70 species; Gornall, 1987; Webb & Gornall, 1989). Although a modest sized family, members of Saxifragaceae have served as important models for studies of autopolyploid speciation (reviewed in Soltis & Soltis, 1999; Segraves & Thompson, 1999), coevolution, and geographic mosaic speciation (e.g., Thompson, 1994; Thompson & Pellmyr, 1992). Members of Saxifra-

[1] This research was supported by DEB 9726225. We thank E. Wells and an anonymous reviewer for helpful comments on the manuscript.

[2] Florida Museum of Natural History and the Genetics Institute, University of Florida, Gainesville, Florida 32611, U.S.A.

[3] Department of Botany, University of Georgia, Athens, Georgia 30602, U.S.A.

[4] Department of Ecology and Evolutionary Biology and the Museum of Natural History and Biodiversity Research Center, University of Kansas, Lawrence, Kansas 66045-2106, U.S.A.

[5] School of Biological Sciences, Washington State University, Pullman, Washington 99164-4236, U.S.A. Current address for Mark Fishbein: Department of Biological Sciences, Mississippi State University, Mississippi State, Mississippi 39762, U.S.A.

[6] Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820-6970, U.S.A.

[7] Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

gaceae have also served recently as an important focal point for studies of gynoecial development and diversification (Kuzoff et al., 2001; Soltis & Hufford, in press).

A vast majority of genera and species in the family is found in the northern hemisphere. Centers of species diversity include western North America, where many of the genera are represented, and mountainous areas of Europe and Asia, where species of *Saxifraga* s. str. abound. A few members of the family are noteworthy in that they occur disjunctly in South America, well separated from other Saxifragaceae. The monotypic *Hieronymusia* is known only from a small area of Argentina and Bolivia (Gornall & Bohm, 1985). The monotypic *Saxifragella* and *Saxifragodes* each occur at the southern tip of South America in Tierra del Fuego. Two species of *Chrysosplenium* also occur in southern South America (Hara, 1957) and several species of *Saxifraga* s. str. section *Saxifraga* are found in the Andes (Webb & Gornall, 1989). The closest relatives of these South American taxa, as well as the possible causes of the South American disjunctions, have, until recently, not been investigated (see Soltis et al., 2001, for an analysis of *Chrysosplenium*).

Generic relationships in Saxifragaceae were long considered poorly understood (reviewed in Spongberg, 1972; Soltis et al., 1991a, 1993, 1996a). In part, this confusion may reflect a high degree of morphological similarity, particularly in vegetative features, among many members of the family. Genera are often distinguished by one or a few pronounced differences in floral and/or fruit morphology. Numerous small genera have been recognized in the family; only nine genera have more than three species (*Lithophragma, Mitella, Astilbe, Rodgersia, Bergenia, Boykinia, Chrysosplenium, Saxifraga* s. str., and *Micranthes*).

Molecular phylogenetic studies have greatly clarified relationships within the family, revealing that the large genus *Saxifraga* is polyphyletic, consisting of two phylogenetically well-separated clades, *Saxifraga* s. str. and *Micranthes* (Soltis et al., 1996a). These studies also revealed a number of well-supported tip clades (Table 1), including the *Boykinia, Heuchera,* and *Darmera* groups, *Astilbe/Saxifragopsis, Leptarrhena/Tanakaea,* and *Peltoboykinia/Chrysosplenium* (Soltis et al., 1993, 1996a; Johnson & Soltis, 1994, 1995, 1998).

Additional studies provided phylogenetic hypotheses of relationships within some of these well-supported tip clades (e.g., Soltis & Kuzoff, 1995; Soltis et al., 1991a, 1996b; Mort & Soltis, 1999; Kuzoff et al., 1999). However, relationships among these tip clades remain poorly understood, despite the use of a combined data set of *rbc*L and *mat*K sequences of approximately 2500 bp (Soltis et al., 1996a). Although branch lengths of the tip clades are moderate in length, those at deep levels are very short. The combination of *mat*K and *rbc*L sequences failed to provide internal support higher than 50% for deep branches within the family (Soltis et al., 1996a; Johnson & Soltis, 1998). Due to the absence of suitable plant material, previous studies also did not clarify the placement of the monotypic genera from South America.

These DNA-based studies provided a phylogenetic framework that permitted initial insights into character evolution in Saxifragaceae, revealing, for example, that multiple origins of several flavonoid compounds had occurred, as well as two independent episodes of extensive polyploidy (e.g., Soltis et al., 1993, 1996a). Inferences regarding character evolution within Saxifragaceae have been compromised, however, by the fact that deep-level relationships remain poorly resolved and weakly supported.

Recent simulation and empirical studies have illustrated the importance of adding both characters and taxa to resolve certain types of difficult phylogenetic problems (e.g., Hillis, 1996; Chase & Cox, 1998; Graybeal, 1998; Soltis et al., 1998; Rannala et al., 1998). In an attempt to resolve relationships at deep levels within Saxifragaceae, we added over 4000 additional characters to our existing DNA data sets. We combine DNA sequences for six DNA regions: two coding regions (*mat*K and *rbc*L) and two spacer regions (*trn*L-*trn*F and *psb*A-*trn*H) representing the plastid genome, as well as ITS and 26S rDNA expansion segments representing the nuclear genome. Using recent collections of *Saxifragella* and *Saxifragodes,* we investigate the relationships of these genera and provide insights into the relationships and origins of the South American disjunct taxa. Using the topologies obtained, we also provide general insights into the geographical and morphological diversification in the family, and present a reclassification of Saxifragaceae.

Materials and Methods

Amplification and Sequencing

We used chloroplast (*mat*K, *rbc*L, *psb*A-*trn*H, *trn*L-*trn*F) and nuclear (ITS, 26S rDNA expansion segments) sequence data in our analyses. The phylogenetic utility of all of these regions has been reviewed (e.g., Soltis & Soltis, 1998). Our sampling strategy was broad, involving 42 representatives of

Table 1. Saxifragaceae, showing genera and the approximate number of species in each. Genera are arranged by well-supported clades based on earlier studies as well as results presented here.

Clade (in Bold)	Approximate Number of Species
Saxifraga s. str. (including *Saxifragella*)	
Saxifraga	350
Peltoboykinia/Chrysosplenium	
Peltoboykinia	1
Chrysosplenium	57
***Darmera* Group**	
Rodgersia	6
Oresitrophe	1
Astilboides	1
Mukdenia	1
Darmera	1
Bergenia	6
***Micranthes* s.l.**	
Micranthes (formerly *Saxifraga sect. Micranthes*)	68
Saxifraga tolmiei[a]	1
Cascadia	1
Saxifragodes[b]	1
***Heuchera* Group**	
Tolmiea	1
Tellima	1
Tiarella	3
Elmera	1
Bensoniella	1
Heuchera	50
Conimitella	1
Mitella[c]	20
Lithophragma	9
***Boykinia* Group**	
Boykinia	7
Bolandra	2
Suksdorfia	2
Jepsonia	3
Telesonix	2
Sullivantia	3
Hieronymusia[d]	1
Leptarrhena/Tanakaea	
Leptarrhena	1
Tanakaea	1
Astilbe/Saxifragodes	
Astilbe	22
Saxifragopis	1
Uncertain Affinity	
Saniculiphyllum[e]	1

[a] *Saxifraga tolmiei* is placed in *Saxifraga* sect. *Merkianeae* by Webb and Gornall (1989), but was previously considered part of section *Micranthes*.

Saxifragaceae (Table 2) and including 28 of the approximately 30 genera. The well-supported tip clades revealed previously are either represented by all constituent genera or by exemplars that span the phylogenetic diversity of each clade. For example, for the large clades *Micranthes* and *Saxifraga* s. str., we included representatives of all of the major subclades revealed by analyses that employed many more representatives (Soltis et al., 1996a; Mort & Soltis, 1999). For the well-supported *Heuchera* group (consisting of nine genera) and *Boykinia* group (consisting of seven genera) four and five genera, respectively, that span the phylogenetic diversity revealed by earlier studies were employed (see Soltis et al., 1991a, 1996b; Soltis & Kuzoff, 1995). For the smaller well-supported subclades, all of the constituent genera were sampled (i.e., *Darmera* group, *Leptarrhena/Tanakaea, Astilbe/Saxifragopsis, Peltoboykinia/Chrysosplenium*).

We also included two monotypic genera (*Saxifragodes, Saxifragella*) from Tierra del Fuego not included in previous phylogenetic analyses due to the difficulty in obtaining material. Thus, only two genera of Saxifragaceae (*Saniculiphyllum, Hieronymusia*), both monotypic and extremely rare and local in occurrence, have not been included in any of our phylogenetic analyses of Saxifragaceae. *Saniculiphyllum* has only been collected once from China (Wu & Ku, 1992) and its current status is unknown. *Hieronymusia* is restricted to remote areas of Argentina and Bolivia. We have been unable to obtain recent collections and material taken from herbarium specimens has yielded only highly degraded DNA. Thus, despite repeated efforts, we have been unable to obtain suitable material of *Hieronymusia*.

Sequences for ITS (Soltis & Kuzoff, 1995; Soltis et al., 1996b; Johnson & Soltis, 1998), *mat*K (Johnson & Soltis, 1995; Soltis et al., 1996a), and *rbc*L (Soltis et al., 1993, 1996a) were reported previously for most taxa. Some 26S rDNA sequences were provided in Kuzoff et al. (1998) and Fishbein et al. (2001). We add here ITS sequences for *Saxifraga hirculus* and *S. fortunei* (*Saxifraga* s. str.), *S. stel-*

←

[b] Inclusion is based on the results of the current study.

[c] *Mitella* is clearly polyphyletic, but the number of genera into which it should be subdivided remains unclear.

[d] Based on morphological and chemical data, the affinities of this genus, for which material has never been obtained, are with the *Boykinia* group.

[e] This genus has only been collected once from China; its relationships are unknown.

Table 2. Species sequenced for the present study and collection data. Two consecutive Genbank numbers are given for each ITS sequence. The first is for ITS-1; following the slash the last two digits of the Genbank accession number for ITS-2 are given. Sequences for which numbers are not given have been previously published (see Materials and Methods): A = Soltis et al. (1993); B = Soltis et al. (1996a); C = Johnson and Soltis (1995); D = Soltis and Kuzoff (1995); E = Johnson and Soltis (1998); F = Soltis et al. (1996b); G = Kuzoff et al. (1998); H = Fishbein et al. (in press). Under Voucher general geographic collection data or area of occurrence are given in parentheses; abbreviations as follows: PNW = Pacific Northwest of North America, N = Norway, C = circumboreal, AK = Alaska, CA = California, IA = Iowa, OR = Oregon, VA = Virginia, WA = Washington.

Group	Taxon	Voucher	*matK*	*rbcL*	*psbA-trnH*	*trnL-F*	ITS	26S
Saxifraga s. str.	*Saxifragella bicuspidata* (Hook.) Engl.	*Arroyo et al. 950914* (Chile)	AF374728	AF374730	AF374733	AF374776	AF374819/20	AF374829
	Saxifraga cymbalaria L.	*Ferguson s.n.*, WS (Turkey)	B	B	AF374734	AF374777	E	AF374830
	Saxifraga mertensiana Bong.	*Grable 11586*, WS (PNW)	B	B	AF374735	AF374778	E	H
	Saxifraga cernua L.	*Brochmann 92-31-22*, OS (N, C)	B	B	AF374736	AF374779	E	AF374831
	Saxifraga fortunei J. D. Hooker	*Soltis 2519*, WS (Japan)	B	B	AF374737	AF374780	AF374821/22	AF374832
	Saxifraga hirculus L.	*Brochmann 93-219*, OS (N, C)	B	B	AF374738	AF374781	AF374823/24	AF374833
	Saxifraga oppositifolia L.	Kew 1975-4135, Cult., K (C)	B	B	AF374739	AF374782	E	AF374834
	Saxifraga rotundifolia L.	BBG 267-94-80-10, Cult., K (Alps)	B	B	AF374740	AF374783	E	AF374835
	Saxifraga scardica Grisebach	Kew 361-84-03742, Cult., K (Greece)	B	B	AF374741	AF374784	E	AF374836
	Saxifraga spathularis Brotero	BBG 001-91-75-10, Cult., K (Spain)	B	B	AF374742	AF374785	E	AF374837
	Saxifraga tricuspidata Rottböll	*Parker s. n.*, WS (AK)	B	B	AF374743	AF374786	E	AF374838
	Saxifraga aizoides L.	*Brochmann 92-78-1*, OS (N, C)	B	B	AF374744	AF374787	E	AF374839
	Saxifraga osloensis Knaben	*Brochmann s. n.*, WS (N)	B	B	AF374745	AF374788	E	AF374840
Darmera	*Rodgersia pinnata* Franch.	Palmengarten Botanical Gardens, Germany, Cult., WS (China)	C	A	AF374748	AF374791	D	AF374841
	Oresitrophe rupifruga Bunge	Beijing Botanical Gardens, China, Cult., WS (China)	B	B	AF374749	AF374792	E	AF374842
	Astilboides tabularis (Hemsl.) Engl.	Palmengarten Botanical Gardens, Germany, Cult., WS (China)	C	A	AF374750	AF374793	E	AF374843
	Mukdenia rosii (Oliver) Koidzumi	University British Columbia, Botanical Gardens, Cult., WS (Korea)	C	A	AF374751	AF374794	E	AF374844
	Darmera peltata (Torr.) Voss	University of California, Berkley, Cult., UC, WS (CA)	C	A	AF374752	AF374795	D	AF374845
	Bergenia cordifolia (Haw.) A. Br.	Komarov Botanical Institute, Russia, Cult, WS (Russia)	C	A	AF374753	AF374796	D	AF374846
Micranthes s. l.	*Saxifraga tolmiei* Torrey & Gray	*WS 32167*, WS (PNW)	B	B	AF374756	AF374799	E	AF374849
	Saxifraga punctata (=nelsonia) D. Don	*Soltis & Soltis 2217* WS (PNW)	B	A	AF374757	AF374800		AF374850
	Saxifraga integrifolia W. J. Hooker	*Soltis & Soltis 2253* (PNW)	C	A	AF374758	AF374801	E	G

Table 2. Continued.

Group	Taxon	Voucher	*matK*	*rbcL*	*psbA-trnH*	*trnL-F*	ITS	26S
	Saxifraga stellaris L.	*Horandl 2703* WS (PNW)	B	AF374732	AF374759	AF374802	AF374827/28	AF374851
Heuchera	*Heuchera micrantha* Dougl.	*Soltis & Soltis 1949*, WS (PNW)	C	A	AF374763	AF374806	D	G
	Elmera racemosa (Wats.) Rydb.	*Soltis & Soltis 2179*, WS (PNW)	C	A	AF374761	AF374804	D	H
	Tolmiea menziesii Torrey & Gray	*Soltis & Soltis 1903*,WS (PNW)	C	A	AF374760	AF374803	D	H
	Tellima grandiflora (Pursh) Dougl.	*Soltis & Soltis 2123*, WS (PNW)	C	A	AF374762	AF374805	D	H
Boykinia	*Telesonix heucheriformis* Rydb.	*Wolf 151*, WS (WY)	C	A	AF374764	AF374807	F	H
	Jepsonia parryii (Torr.) Small	*Rieseberg 1110*, WS (CA)	C	A	AF374765	AF374808	F	H
	Bolandra oregona Wats.	*Grable 11668*, WS (OR)	C	A	AF374766	AF374809	F	H
	Boykinia rotundifolia Parry	*Gornall 0101*, UBC (CA)	C	A	AF374767	AF374810	F	H
	Sullivantia oregana Wats.	*Gornall 11214*, WS (OR)	C	A	AF374768	AF374811	F	H
Astilbe/Saxifragopsis	*Astilbe microphylla* Knoll.	Botanical Gardens, Univ. Tokyo, Cult., WS (Japan)	C	A	AF374771	AF374814	E	H
	Saxifragopsis fragarioides (Greene) Small	UCBG 82-1325, UC (CA)	AF374729	A	AF374772	AF374815	E	H
Leptarrhena/Tanakaea	*Leptarrhena pyrolifolia* (D. Don) R. Br.	*Soltis & Soltis 2237*, WS (AK)	C	A	AF374769	AF374812	F	H
	Tanakaea radicans Franch.	Nikko Botanical Garden, Japan, WS (Japan)	C	A	AF374770	AF374813	F	H
Cascadia/Saxifragodes	*Cascadia nutallii* (Small) A. M. Johnson	University of Washington 3466, WTU (OR)	B	A	AF374755	AF374798	E	H
	Saxifragodes albowiana (Kurtz) D. M. Moore	*Arroyo et al. 941179*, WS (Chile)	AF374729	AF374731	AF374754	AF374797	AF374825/26	AF374847
Chrysosplenium/Peltoboykinia	*Chrysosplenium iowense* Rydb.	*Wendel s.n.*, WS (IA)	C	A	AF374747	AF374790	E	H
	Peltoboykinia tellimoides (Maxim.) Hara	Nikko Botanical Garden, Japan, Ws (Japan)	C	A	AF374746	AF374789		G
Outgroup	*Ribes aureum* Pursh	*Soltis & Soltis 2220*, WS (WA)	C	A	AF374773	AF374816	E	H
	Pterostemon rotundifolius Ramirez	Jordan s.n., WS (Mexico)	B	A	AF374774	AF374817	E	H
	Itea virginica L.	*D. M. E. Ware 94*, WILI (VA)	C	A	AF374775	AF374818	E	H

laris (*Micranthes*), *Saxifragodes*, and *Saxifragella*. For *rbc*L, new sequences were generated for *Saxifragodes*, *Saxifragella*, and *Saxifraga stellaris*; for *mat*K new sequences were produced for *Saxifragodes* and *Saxifragella*. In addition, three new data sets were constructed: 26S rDNA expansion segment sequences; *trn*L-*trn*F and *psb*A-*trn*H spacer sequences. A majority of the 26S rDNA expansion segment sequences, as well as all *trn*L-*trn*F and *psb*A-*trn*H sequences employed in this study are first reported here (Table 2).

cpDNA sequences. In addition to the chloroplast genes *mat*K and *rbc*L, previous studies have indicated the value of several spacer regions in the chloroplast genome for resolving relationships at the species and generic levels (reviewed in Soltis & Soltis, 1998). Two such regions are the *trn*L–*trn*F intergenic spacer and its adjacent intron (Taberlet et al., 1991; Gielly & Taberlet, 1994, 1996) and the *psb*A–*trn*H spacer region (Sang et al., 1997).

Chloroplast regions were amplified as follows: primers *psb*AF/*trn*HR (for the spacer region *pbs*A-*trn*H; Sang et al., 1997); universal primers C/F (for the spacer region *trn*L-*trn*F; Taberlet et al., 1991); *mat*K-3914F/*psb*AR (for *mat*K; Johnson & Soltis, 1994); Z1–3′ *rbc*L (for *rbc*L, Olmstead et al., 1993). For *trn*L-*trn*F and *psb*A-*trn*H, the same primers used for PCR amplification were also employed for DNA sequencing. For *rbc*L, the sequencing primers used were Z1, 346R, 674R, 674, 894R, 894, 1351R, and 3′ *rbc*L (Olmstead et al., 1993). For *mat*K, the sequencing primers used were 1412F, 1470R, 1470F (Johnson & Soltis, 1994, 1995). Automated sequencing of *mat*K, *trn*L-*trn*F, and *psb*A-*trn*H followed the general methods of Mort et al. (2001, in press); automated sequencing of *rbc*L followed Lledó et al. (1998).

Nuclear sequences. Amplification and sequencing of ITS employed the general approaches provided in Soltis and Soltis (1997), Kuzoff et al. (1999), and Mort et al. (in press). For ITS amplification, we used primers N-nc18S10 and C26A (Wen & Zimmer, 1996). These PCR primers subsequently served as sequencing primers.

26S rDNA sequences were amplified via PCR using the primers N-nc 26S1 and 3331R following Kuzoff et al. (1998). Partial 26S rDNA sequences were employed, consisting of 950 bp from the 5′ end of the gene, as well as 250 bp from the 3′ end of the gene. These two regions contain the four expansion segments D1, D2, D3, and D12 and encompass over 70% of the variation present in all 12 of the 26S rDNA expansion segments (Kuzoff et al., 1998). The forward sequencing primers employed were: N-nc26S1, N-nc26S3, N-nc26S5, and N-nc26S14; the reverse primers used were 268R, 641R, 950R, and 3331R (see Kuzoff et al., 1998).

ALIGNMENT

The *mat*K, *rbc*L, and 26S rDNA sequences were easily aligned visually. Several indels (all in multiples of three base pairs) were present in the *mat*K data set; these have been reported and discussed previously (Johnson & Soltis, 1994, 1995; Soltis et al., 1996a). The *trn*L-*trn*F, and *psb*A-*trn*H, and ITS sequences contained regions that were more prone to insertion and deletion. We will first discuss alignment of the *trn*L-*trn*F and *psb*A-*trn*H regions; alignment of ITS is discussed separately below.

Portions of the *psb*A-*trn*H and *trn*L-*trn*F regions were difficult to align; the *psb*A-*trn*H region, in particular, was problematic. These two cpDNA regions were aligned with CLUSTAL X using the default settings (Thompson et al., 1997); we subsequently modified and checked these alignments by eye. This approach enabled us to align and use most of the *trn*L-*trn*F region (four regions totaling 184 bp were ultimately removed from all phylogenetic analyses); in contrast, we found much of the *psb*A-*trn*H region so difficult to align that we eliminated much of this region; our analyses involved only 221 bp of 658 total bp.

For ITS we first employed a global alignment strategy in which the sequences for all taxa were aligned using CLUSTAL X, again using the default settings. Following this initial alignment, further adjustments were made by eye. However, we considered this a "rough" alignment. Sequences of taxa representing the well-supported clades within Saxifragaceae (e.g., *Boykinia* and *Heuchera* groups, *Saxifraga* s. str., *Micranthes*, *Leptarrahena*/*Tanakaea*) were easily aligned, whereas alignment across groups was more problematic. We noted that global approaches often erred by compromising the alignments of closely related taxa within well-supported groups in an effort to produce alignments between more heterogeneous sequences.

We therefore used a second approach employed previously for ITS across the entire Saxifragaceae (Johnson & Soltis, 1998). Alignments were first constructed within well-supported clades (e.g., within the *Boykinia* group, *Heuchera* group, *Chrysosplenium*/*Peltoboykinia*, etc.); this was often possible by eye, although CLUSTAL was also employed. Global alignments were subsequently constructed using CLUSTAL and then adjusted manually with extensive efforts made to preserve these local alignments. The initial alignments constructed for Saxifragaceae (Johnson & Soltis, 1998)

employed CLUSTAL W (Thompson et al., 1994) and were based on extensive comparisons of different pairwise alignment dendrograms derived by using different gap-opening (GOP) penalties and gap-extension (GEP) penalties. For example, we compared 10 different GOP and GEP penalties in Soltis et al. (1996b). Earlier studies of ITS in Saxifragaceae (Soltis & Kuzoff, 1995; Soltis et al., 1996b; Johnson & Soltis, 1998; Kuzoff et al., 1999) suggested that because of the disparity of length of ITS across Saxifragaceae, relaxed GOP and GEP penalties should be used; lower values make it easier to initiate or extend gaps. Gaps were therefore preferred to maximize the alignment of regions across the well-defined clades of Saxifragaceae. We constructed five alignments with this more laborious approach. Thus, we experimented with a total of six different alignments for ITS—one relatively rough alignment (noted above) plus five additional alignments that we considered more rigorous.

In addition to the use of multiple alignments, we also experimented extensively with the removal of portions of our ITS alignments that were difficult to align using the ELIMINATE (= ZAP) command available in PAUP*. In constructing these "culled" data sets, five difficult to align regions were identified. Initial analyses employed all base positions, with each subsequent analysis removing one more area of potentially problematic alignment; in the final culled data set, all five areas of ambiguous alignment were removed. These difficult-to-align regions range in length from 20 to 50 bp; all five problematic segments totaled 165 total bp.

PHYLOGENETIC ANALYSES

Parsimony analyses were conducted using a test version (4.0d60) of PAUP* (Swofford, 1999) for: (1) all individual DNA regions; (2) all cpDNA regions combined; (3) both nuclear regions combined; (4) all six DNA regions combined (data sets are available on our website: http://www.flmnh.ufl.edu/soltislab). We conducted parsimony analyses on six different ITS alignments. We also conducted parsimony analyses on six different combined ITS + 26S rDNA data sets, as well as on six different combined data sets for all genes that involved each of these six different ITS alignments. Gaps were treated as missing data and scored as ambiguous ("?"). We conducted heuristic searches with MULPARS, and TBR branch swapping. We searched for multiple islands of most-parsimonious trees (Maddison, 1991) using 1000 replications of RANDOM taxon addition. Internal support for clades was assessed using the bootstrap with 1000 random taxon addition replicates, MULPARS, and TBR branch swapping saving 10 trees per replicate.

Table 3. Likelihoods (ln transformed), proportion of invariant sites, and Γ values for data sets analyzed with ML and estimated values of evolutionary parameters of ML trees for three data partitions.

Data set	−ln of MP tree used to estimate model parameters	Proportion of invariant sites	Γ	−ln of final ML tree
*mat*K	7,738.28	0.003806	1.069204	7,736.35
all cpDNA	22,316.73	0.35573	1.020555	22,136.73
both nuclear	14,273.98	0.439973	0.676933	14,263.06
all six genes	39,034.46	0.403566	0.61397	37,284.03

ML analyses were done using PAUP 4.0b2 (Swofford, 1999). We used a general time-reversible model of nucleotide substitution (GTR; the same as REV of Yang, 1994) with a fixed proportion of invariant sites and with gamma-distributed rate variation among sites free to vary (I + G; Gu et al., 1995). Likelihood scores for each of the most parsimonious trees from each of the above data sets were estimated along with GTR + I + G substitution parameters (Table 3). A neighbor-joining tree using the JC69 model of molecular evolution was obtained using PAUP*, and commands from MODELTEST 3.0 (Posada & Crandall, 1998) for each of the above data sets were used for model selection. MODELTEST 3.0 (Posada & Crandall, 1998) was used to determine the substitution model that best fits the data. MODELTEST 3.0 (Posada & Crandall, 1998) uses likelihood scores, calculated using PAUP*, for 56 models of molecular evolution (JC to GTR + I + G) to compare the different nested models of molecular evolution. MODELTEST 3.0 tests the nested models of molecular evolution by calculating a likelihood ratio test statistic and p-value assuming a c2 distribution with q degrees of freedom. In addition to the likelihood ratio test, MODELTEST 3.0 also calculates the Akiake information criterion (AIC), which compares different models of molecular evolution but does not require the models to be nested. The AIC selects models for good fit; however, it penalizes models for unnecessary parameters. In all cases, the GTR + I + G substitution model fit the data significantly better than any of the other substitution models analyzed here. Likelihood scores for each of the most parsimonious trees from each of the above data sets were estimated along with GTR + I + G substitution parameters (Table 3). A single MP tree with

the lowest likelihood score was selected from each of the above data sets and used to estimate parameters for the GTR + I + G model. ML searches used heuristic search strategies with 100 random taxon additions for the combined six gene data set, and 10 random taxon additions for the remaining data sets, followed by TBR (tree bisection reconnection) branch swapping, holding 5 trees at each step. Two ML searches were done on each of the data sets except for the ITS data sets, which only had a single ML search. The second ML search used model parameters estimated from the tree obtained from the first ML search and used the same heuristic search strategy as employed in the first ML analysis (Table 3).

Based on phylogenetic analyses encompassing all major groups of angiospserms (e.g., Savolainen et al., 2000; Soltis et al., 2000), as well as studies focused on Saxifragales (Fishbein et al., 2001), the closest relatives of Saxifragaceae are clear: Grossulariacae, Iteaceae, and Pterostemonaceae. *Ribes*, *Itea*, and *Pterostemon* therefore serve as outgroups. *Ribes* is characterized by a long branch in some of the individual data sets; for some ITS alignments, inclusion of *Ribes* impacted the placement of one clade. As a result, for all data sets we also conducted parsimony analyses with just *Itea* and *Pterostemon* as outgroups.

CONGRUENCE

The issue of when, and if, separate data sets should be combined for phylogenetic analysis has been extensively debated and reviewed (e.g., Bull et al., 1993; de Queiroz et al., 1995; Miyamoto & Fitch, 1995, Mason-Gamer & Kellogg, 1996; Seelanen et al., 1997; Johnson & Soltis, 1998). As we and others have frequently done, we employed a conditional combination approach (see Huelsenbeck et al., 1996), following the general strategy of Mason-Gamer and Kellogg (1996) and Johnson and Soltis (1998).

We divided the data sets into two process partitions: nuclear data sets (ITS and 26S rDNA); and cpDNA data sets (*rbc*L, *mat*K, *trn*L-*trn*F, *psb*A-*trn*H). Because the chloroplast genome is inherited as a unit and not subject to recombination, we treated them as a single linkage partition (reviewed in Soltis & Soltis, 1998). We initially conducted separate analyses of each cpDNA set; we similarly conducted separate analyses of the two nuclear data sets. The resultant topologies were then compared; we searched for instances of well-supported alternative placements, or hard incongruence (Seelanen et al., 1997). We employed a bootstrap value of 70% as a cutoff for assessing strong conflict among data sets, following the approach of Mason-Gamer and Kellogg (1996). We also explored heterogeneity among data sets using the partition homogeneity test (Farris et al., 1995) as implemented in PAUP (Swofford, 1999).

RESULTS

CPDNA GENES

We first present the results for the individual genes, the aligned lengths of which are: *mat*K 1078 bp; *rbc*L 1514 bp; *psb*A-*trn*H 658 bp; *trn*L-*trn*F 1321 bp. To save space we depict only the combined cpDNA tree; the individual gene trees are available on our website (http://flmnh.ufl.edu/soltislab).

rbc*L*. Analyses of *rbc*L alone yielded five shortest trees of length 462 (CI = 0.602; RI = 0.758) and revealed two major clades within Saxifragaceae (tree not shown). A strongly supported (98%) *Saxifraga* s. str. (including *Saxifragella*) is present; several relationships within *Saxifraga* s. str. are also well supported. The second major clade, hereafter the heucheroid clade, comprises all taxa other than *Saxifraga* s. str. and does not receive support greater than 50%. Based on *rbc*L sequences, *Saxifragodes* is part of the heucheroid clade, where it appears as the strongly supported (100%) sister of *Cascadia*. Other groups with support greater than 50% in the heucheroids include *Astilbe/Saxifragopsis* (70%), *Boykinia* group (70%), *Leptarrhena/Tanakaea* (77%), *Chrysosplenium/Peltoboykinia* (52%), *Micranthes* (96%), and *Heuchera* group (96%).

mat*K*. Parsimony analysis of *mat*K alone resulted in five shortest trees of length 1064 (CI = 0.669; RI = 0.764). Two major clades of Saxifragaceae s. str. are again revealed (tree not shown). *Saxifraga* s. str. (including *Saxifragella*) forms a strongly supported (100%) clade. In contrast to the results of *rbc*L, however, *Saxifragodes* appears as the immediate sister to *Saxifraga* s. str. (61% bootstrap support), rather than with *Cascadia* in the heucheroid clade. The remaining Saxifragaceae form a second clade (without support greater than 50%) that corresponds to the heucheroid clade, excluding *Saxifragodes*. Several well-supported clades are also evident within *Saxifraga* s. str., as well as within the heucheroids. Within the latter clade, the following receive support greater than 50%: *Astilbe/Saxifragopsis* (95%), *Boykinia* group (100%), *Leptarrhera/Tanakaea* (100%), *Peltoboykinia/Chrysosplenium* (77%), *Micranthes* s. str. (99%), a *Micranthes* s.l. clade (96%) that also includes *Cascadia* and *Saxifraga tolmiei* (a *Micranthes* s.l.

clade is also revealed by other DNA regions, and includes *S. tolmiei*, but not *Cascadia*; see below), *Darmera* group (65%), and *Heuchera* group (100%). Relationships among these groups do not receive support greater than 50%.

Because *Saxifragodes* possesses a long branch with *mat*K and its phylogenetic position differs from that obtained with *rbc*L (as well as from other regions), we also analyzed the *mat*K data set with MLE and recovered the same topology as was found with parsimony (tree not shown). We also re-sequenced a second DNA isolate of *Saxifragodes* to check for sequencing errors but obtained an identical sequence.

trn*L*-trn*F*. This region is prone to insertion and deletion in Saxifragaceae. Most of the indels are greater in length than just one or two bp, with many long indels present that are unique to individual taxa. We experimented with the removal of three of the four difficult to align regions (21, 50, and 52 bp in length). The topological impact of these regions was minimal. One of the regions had no impact on the topologies obtained; when the two longer, hard to align regions were removed, more shortest trees were obtained (e.g., six vs. three in one analysis) with lower resolution achieved among the major clades within the heucheroids in the strict consensus trees. In all cases a major dichotomy was observed within the Saxifragaceae–*Saxifraga* s. str. and the heucheroid clade.

Parsimony analyses of *trn*L-*trn*F yielded 252 trees of length 852 (CI = 0.754; RI = 0.744). Two major clades are evident in Saxifragaceae: a well-supported *Saxifraga* s. str. that includes *Saxifragella* (100%); the heucheroid clade, which receives support less than 50% (tree not shown). Other well-supported groups revealed by *trn*L-*trn*F sequence data include: *Astilbe/Saxifragopsis* (80%), *Micranthes* (77%), *Micranthes* + *Saxifraga tolmiei* (97%), *Cascadia/Saxifragodes* (80%), *Heuchera* group (100%), *Telesonix* with *Leptarrhena/Tanakaea* (84%), a relationship that does not appear in any other analyses. The *Boykinia* group receives weak support (54%) but does not include *Telesonix*. The *Darmera* group is monophyletic but does not receive support greater than 50%. Two subclades within the *Darmera* group each appear with bootstrap support greater than 50%: *Oresitrophe/Mukdenia/Bergenia* (65%) and *Rodgersia/Astilboides/Darmera* (73%). There is considerable support for relationships within *Saxifraga* s. str.

*psb*A-*trn*H. A large portion of this spacer region was extremely difficult to align across Saxifragaceae and, hence, was ultimately excluded; only 221 of 658 bp were employed. Analysis of this region yielded over 9000 trees of length 292 (CI = 0.596; RI = 0.760); the analysis was stopped prior to completion. Branch lengths are very short and little resolution and support are evident. A strongly supported *Saxifraga* s. str. clade is present (92%) that includes *Saxifragella*; several relationships within *Saxifraga* s. str. also receive support greater than 50%. Only a few other clades receive support greater than 50%: *Cascadia/Saxifragodes* (81%), *Micranthes* (83%), *Micranthes* + *Saxifraga tolmiei* (89%), and a subset of the *Heuchera* group (*Elmera*, *Tellima*, and *Heuchera*; 51%). Groups that are monophyletic and well supported with other genes (e.g., *Heuchera* group, *Boykinia* group, *Chrysosplenium/Peltoboykinia*, *Astilbe/Saxifragopsis*) are not retrieved with the *psb*A-*trn*H data set.

COMPARISON OF cpDNA TREES

Comparison of the topologies obtained for individual regions suggests strong overall similarities. Significantly, three of the DNA regions sequenced, *mat*K, *rbc*L, and *trn*L-*trn*F, indicate a major split within the family between *Saxifraga* s. str. and the heucheroid clade. *Saxifraga* s. str. and *Micranthes* each receive strong support (greater than 90%) with all four regions. *Cascadia/Saxifragodes* are a strongly supported sister group with all cpDNA genes, except *mat*K. Several other clades also receive support greater than 50% in three of the four cpDNA data sets (all but *psb*A-*trn*H): *Astilbe/Saxifragopsis*, *Peltoboykinia/Chrysosplenium*, and the *Heuchera* group. Three of the cpDNA data sets (all but *psb*A-*trn*H) also suggest a close relationship between *Leptarrhena/Tanakaea* and the *Boykinia* group, although only *mat*K does so with support greater than 50%. Two of the cpDNA data sets (*mat*K and *trn*L-*trn*F) also indicate the monophyly of the *Darmera* group.

Contradictory clades among the cpDNA-based topologies each received less than 70% bootstrap support. Hence, there are no instances of hard incongruence among these trees. The most obvious incongruence among cpDNA regions involves the different placement of *Saxifragodes*. With *mat*K, the genus appears as sister to *Saxifraga* s. str. with weak support (64%), whereas the remaining cpDNA trees place *Saxifragodes* with *Cascadia* (with strong support).

COMBINED cpDNA DATA SET

The total combined aligned length of the four cpDNA regions employed is 4559 bp, with 621 bp excluded yielding a total of 3938 bp used in most analyses. With *Ribes* removed as an outgroup, phylogenetic analysis of a combined data set of all four

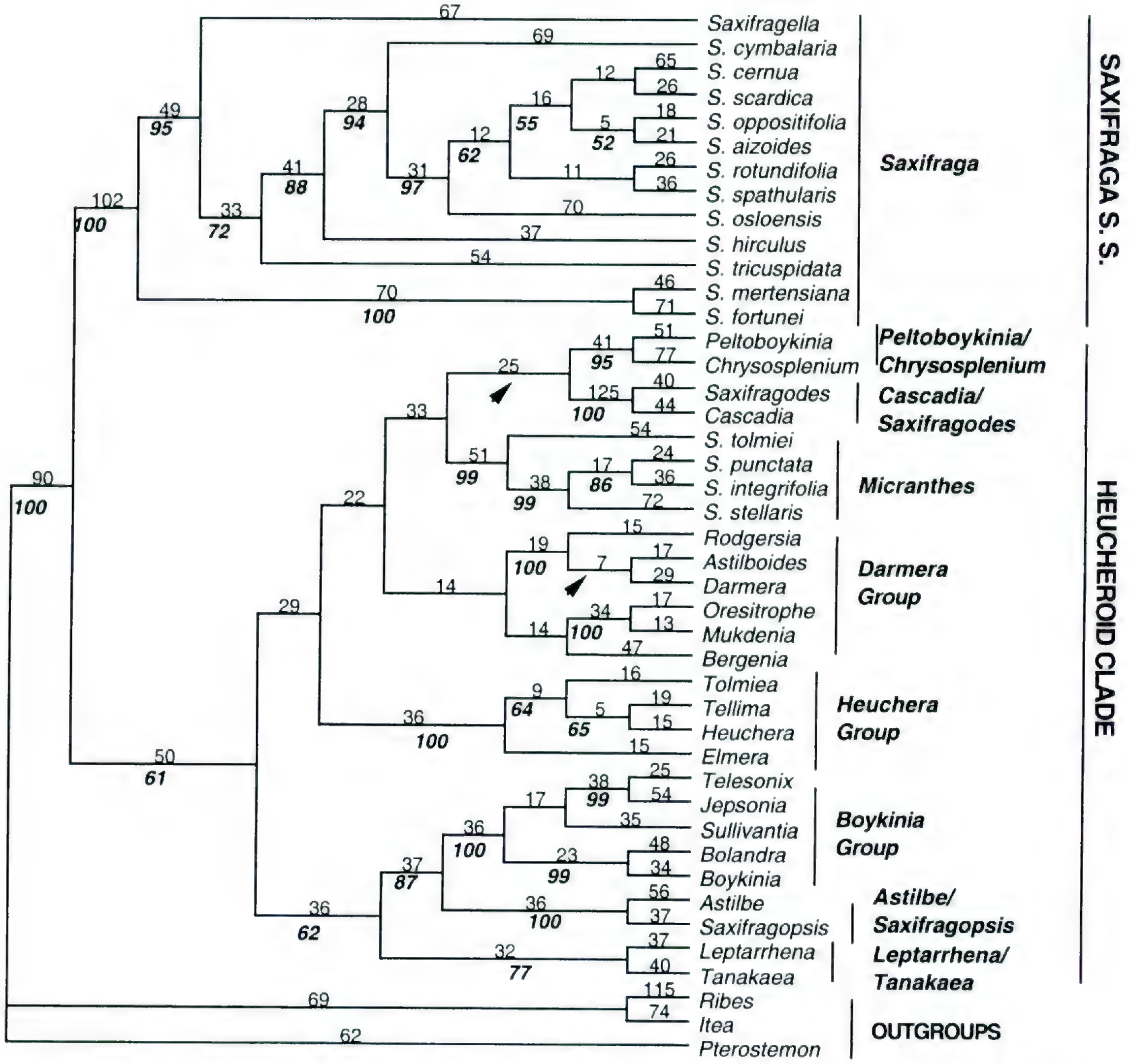


Figure. 1. One of three shortest trees resulting from parsimony analysis of combined cpDNA data sets (*rbc*L, *mat*K, *trn*L-*trn*F, *psb*A-*trn*H); length = 2962, CI = 0.624, RI = 0.695. Percentages below branches are bootstrap values; numbers above branches are the number of base substitutions. Arrows indicate branches that collapse in the strict consensus of all shortest trees.

chloroplast genes resulted in one shortest tree of length 3031 steps (CI = 0.622, RI = 0.689). With *Ribes* included (Fig. 1), three trees of length 2962 are obtained (CI = 0.624; RI = 0.695). The strict consenus of these trees reveals less resolution at deep levels in the heucheroid clade than obtained when *Ribes* is omitted.

Based on cpDNA data (Fig. 1), the family consists of two major clades: (1) a strongly supported (100%) *Saxifraga* s. str. (plus *Saxifragella*) and (2) the heucheroid clade, which receives support less than 50%. Many relationships within *Saxifraga* s. str. are well supported. *Saxifraga mertensiana* and *S. fortunei* form a clade (100% support) that is sister to the remaining members of the clade (100% support). The next branching member is *Saxifragella*, which is sister to the remainder of the clade, which is again well supported (100%). *Saxifraga tricuspidata* then follows as sister to a well-supported (98%) core group of taxa.

The heucheroid clade consists of a number of groups that receive support greater than 50%. *Astilbe/Saxifragopsis* form a strongly supported (100%) sister group; they in turn are sister (but without support greater than 50%) to two other well-supported groups: *Boykinia* group (96%) and *Leptarrhena/Tanakaea* (100%). The sister group relationship between *Leptarrhena/Tanakaea* and the *Boykinia* group receives bootstrap support of 71%. The remainder of the heucheroid clade consists of

Peltoboykinia/Chrysosplenium (82%); the *Darmera* group (67%); *Cascadia/Saxifragodes* (53%); *Micranthes* (100%), *Micranthes* + *S. tolmiei* (55%), and the *Heuchera* group (100%). None of the relationships among these tip clades within the heucheroids receives support greater than 50%.

INDIVIDUAL NUCLEAR GENES

The length of what we have termed the "rough" ITS alignment is 641 bp; the length of each of the remaining ITS alignments employed is 687 bp (315 bp for ITS-1; 372 bp for ITS-2). The aligned 26S rDNA data set is 1191 bp. To save space we depict only the combined ITS + 26S rDNA tree; the individual gene trees are available on our website (http://www.flmnh.ufl.edu/soltislab).

ITS. As noted, we employed six ITS alignments, one that we considered relatively rough, and five that were constructed in a more laborious fashion, taking into consideration the local alignment within the well-supported clades. We first compared these six different ITS alignments with all base pairs included and subsequently experimented with removing regions that were hard to align. These initial analyses revealed that the placement of *Cascadia/Saxifragodes* is sensitive to outgroup selection in three of the six alignments. When all three outgroup taxa are employed, *Cascadia/Saxifragodes* appear as sister to the remainder of Saxifragaceae in five of six alignments. With one alignment, *Cascadia/Saxifragodes* appear within the heucheroid clade close to *Micranthes*. However, with *Ribes* removed, the topologies obtained for three of the six alignments change; following parsimony analysis *Cascadia/Saxifragodes* now appear within the heucheroid clade close to *Micranthes*. The branch leading to *Cascadia/Saxifragodes* is very long in all of the ITS trees, as is the branch to *Ribes* (the number of steps on the branch to *Ribes* is the largest in the entire topology). To examine the possibility that long branch attraction might be impacting the placement of *Cascadia/Saxifragodes*, we applied MLE to the three alignments in which the position of *Cascadia/Saxifragodes* changed with the removal of *Ribes*. For all three of these alignments, with all three outgroup taxa employed, MLE places *Cascadia/Saxifragodes* with *Micranthes*, thus revealing topologies comparable to those achieved with parsimony with *Ribes* removed. We therefore discuss below the parsimony results with *Ribes* removed.

With all ITS sites included, two basic topologies were obtained following parsimony analyses. Four alignments revealed two major clades in Saxifragaceae, *Saxifraga* s. str. and the heucheroid clade (tree lengths = 2289, 2274, 2274, 2260). One of the two remaining alignments resulted in a very similar topology (tree length = 2285), differing primarily in the placement of *Cascadia/Saxifragodes*. Rather than appearing within the heucheroid clade close to *Micranthes* as with the first four alignments, this sister pair appears instead as sister to all other Saxifragaceae s. str. The remainder of the family still comprises two clades, *Saxifraga* s. str. and the heucheroid clade (but in this case minus *Cascadia/Saxifragodes*). For these five similar alignments, the CI ranges from 0.466 to 0.469; RI ranges from 0.620 to 0.625. In contrast, the rough alignment resulted in 26 shortest trees of noticeably greater length (2651 steps); (CI = 0.390; RI = 0.575), but again resulted in a topology similar to those noted above. *Cascadia/Saxifragodes* appear as sister to the remainder of Saxifragaceae. The remainder of the family consists of a trichotomy: (1) *Saxifraga* s. str.; (2) *Micranthes*; (3) the remaining genera of the heucheroid clade.

The remaining differences among the trees resulting from the six alignments used are slight. For example, *Peltoboykinia* and *Chrysosplenium* do not always appear as sister taxa, the *Darmera* group is not always monophyletic, and relationships of members of the *Darmera* group to other members of the heucheroid clade vary.

We describe here the topology of the alignment that provided the shortest trees (and highest CI and RI values). Parsimony analysis of this "best" ITS alignment resulted in one shortest tree of length 2260 (CI = 0.469; RI = 0.625) and revealed two major clades within Saxifragaceae, each with support greater than 50%: *Saxifraga* s. str., including *Saxifragella* (100%), and the heucheroid clade (58%). There is considerable support for relationships within *Saxifraga* s. str. Within the heucheroid clade, the following receive support greater than 50%: *Peltoboykinia/Chrysosplenium* (79%), *Micranthes* (90%), *Micranthes* + *Saxifraga tolmiei* (99%), *Saxifragodes/Cascadia* (100%), *Heuchera* group (100%), *Leptarrhena/Tanakaea* (76%), *Boykinia* group (88%), and *Astilbe/Saxifragopsis* (98%). There is also weak support (54%) for a sister group relationship between *Chrysosplenium/Peltoboykinia* and *Micranthes* + *S. tolmiei*; and weak support (53%) for a clade of *Leptarrhena/Tanakaea*, *Boykinia* group, and *Astilbe/Saxifragopsis*.

Removal of difficult-to-align regions had minor impact on the topology. In most instances, two major clades are still apparent (*Saxifraga* s. str. and the heucheroid clade). In some cases, *Saxifragodes/Cascadia* appear as sister to these two clades. With all alignment-ambiguous regions removed, we

obtained 16 trees of length 1626 (CI = 0.462; RI = 0.628). The strict consensus again reveals two major clades, *Saxifraga* s. str. and the heucheroid clade. Relationships within *Saxifraga* s. str. are only slightly changed from those in which all bp are included, involving only the relationships of *S. spathularis* and *S. rotundifolius*. Many of the relationships found within the heucheroid clade are also identical to those obtained with all bp included. With these regions removed, however, the *Darmera* group is not monophyletic, *Leptarrhena* and *Tanakaea* do not appear as sister taxa, and relationships within the *Boykinia* group are not completely resolved; relationships among the *Boykinia* group, *Leptarrhena/Tanakaea*, and *Astilbe/Saxifragopsis* are unclear. These findings suggest that there is some loss of phylogenetic signal when difficult to align regions are removed; furthermore, that impact is observed even on relationships that are generally well resolved.

26S rDNA. Parsimony analysis of 26S rDNA sequences resulted in 12 trees each of length 789 (CI = 0.450; RI = 0.661). Many of the same well-supported clades retrieved with other genes are again monophyletic and receive bootstrap support greater than 50%: *Saxifraga* s. str. (including *Saxifragella*; 100%), *Micranthes* (99%), *Micranthes* + *Saxifraga tolmiei* (81%), *Peltoboykinia/Chrysosplenium* (96%), *Saxifragodes/Cascadia* (100%), *Boykinia* group (60%), *Astilbe/Saxifragopsis* (74%), and *Heuchera* group (82%). There is also considerable resolution and support of relationships within *Saxifraga* s. str. The monophyly of the *Darmera* group is not supported by 26S rDNA, although the two component subclades each receive bootstrap support greater than 50% (*Oresitrophe/Mukdenia*—89%; *Rodgersia, Astilboides, Darmera*—75%). As was observed with ITS, *Astilbe/Saxifragopsis* appear as the sister to the *Boykinia* group with moderate support (78%); *Tanakaea* and *Leptarrhena*, which form a grade rather than a clade, are successive sisters to the *Astilbe/Saxifragopsis* + *Boykinia* group with 57% bootstrap support. The number of base substitutions observed on most branches of the 26S rDNA trees is low with very little resolution at deep levels.

COMPARISON OF ITS AND 26S rDNA TREES

Comparison of the ITS and 26S rDNA topologies suggests strong overall similarities. There are no examples of hard incongruence involving these two data sets. Both topologies show many of the same clades, including *Saxifraga* s. str. (and many of the subclades therein), *Peltoboykinia/Chrysosplenium*, *Micranthes*, *Cascadia/Saxifragodes*, *Astilbe/Saxifragopsis*, *Heuchera* group, *Boykinia* group, *Oresitrophe/Bergenia*, and *Rodgersia/Astilboides/Darmera*. The differences observed in the 26S rDNA trees when compared to those retrieved with ITS likely reflect lower signal with 26S rDNA expansion segments.

COMBINED NUCLEAR DATA SETS

We combined each of the six different ITS alignments with the 26S rDNA data set. The length of the rough ITS alignment + 26S rDNA data set is 1832 bp; the length of the remaining five ITS alignments + 26S rDNA is 1878 bp. We describe the topology resulting from the combination of the ITS alignment that yielded the shortest trees (with all base pairs included, as well as with difficult to align regions removed). Parsimony analysis of the combined 26S rDNA+ ITS data set (Fig. 2) resulted in four shortest trees of length 3217 (CI = 0.455 ; RI = 0.621). Our MLE analysis of the same combined data set yielded a topology identical to one of the shortest trees obtained using parsimony.

Based on both parsimony and MLE analyses, two major clades are revealed in Saxifragaceae: (1) a strongly supported *Saxifraga* s. str. (plus *Saxifragella*; 100%) clade; (2) a weakly supported heucheroid clade (61%). Within *Saxifraga* s. str. a strongly supported *S. mertensiana/S. fortunei* (100%) is sister to the remainder of the clade; *Saxifragella* is the subsequent sister to the remainder of the clade, followed, in order, by *S. tricuspidata*, *S. hirculus*, and then *S. cymbalaria*; all of these initial branches receive support greater than 70%. The remaining members of *Saxifraga* s. str. form a well-supported clade (97%).

Within the heucheroid clade, a weakly supported (62%) subclade is present of *Leptarrhena/Tanakaea* (77%) as sister to a clade (87%) of *Astilbe/Saxifragopsis* (100%) plus the *Boykinia* group (100%). The remaining members of the heucheroids comprise several subclades, most with support greater than 50%: (1) *Peltoboykinia/Chrysosplenium* (95%); (2) *Saxifragodes/Cascadia* (100%); (3) *Micranthes* (99%); (4) *Micranthes* + *Saxifraga tolmiei* (99%); (5) *Heuchera* group (100%). The *Darmera* group forms a clade but does not receive support greater than 50%. Relationships among these subclades do not receive support greater than 50%.

CONGRUENCE BETWEEN NUCLEAR AND cpDNA TOPOLOGIES

There is a high degree of overall similarity between the combined cpDNA tree and the combined nuclear tree. Both suggest a major split in the family

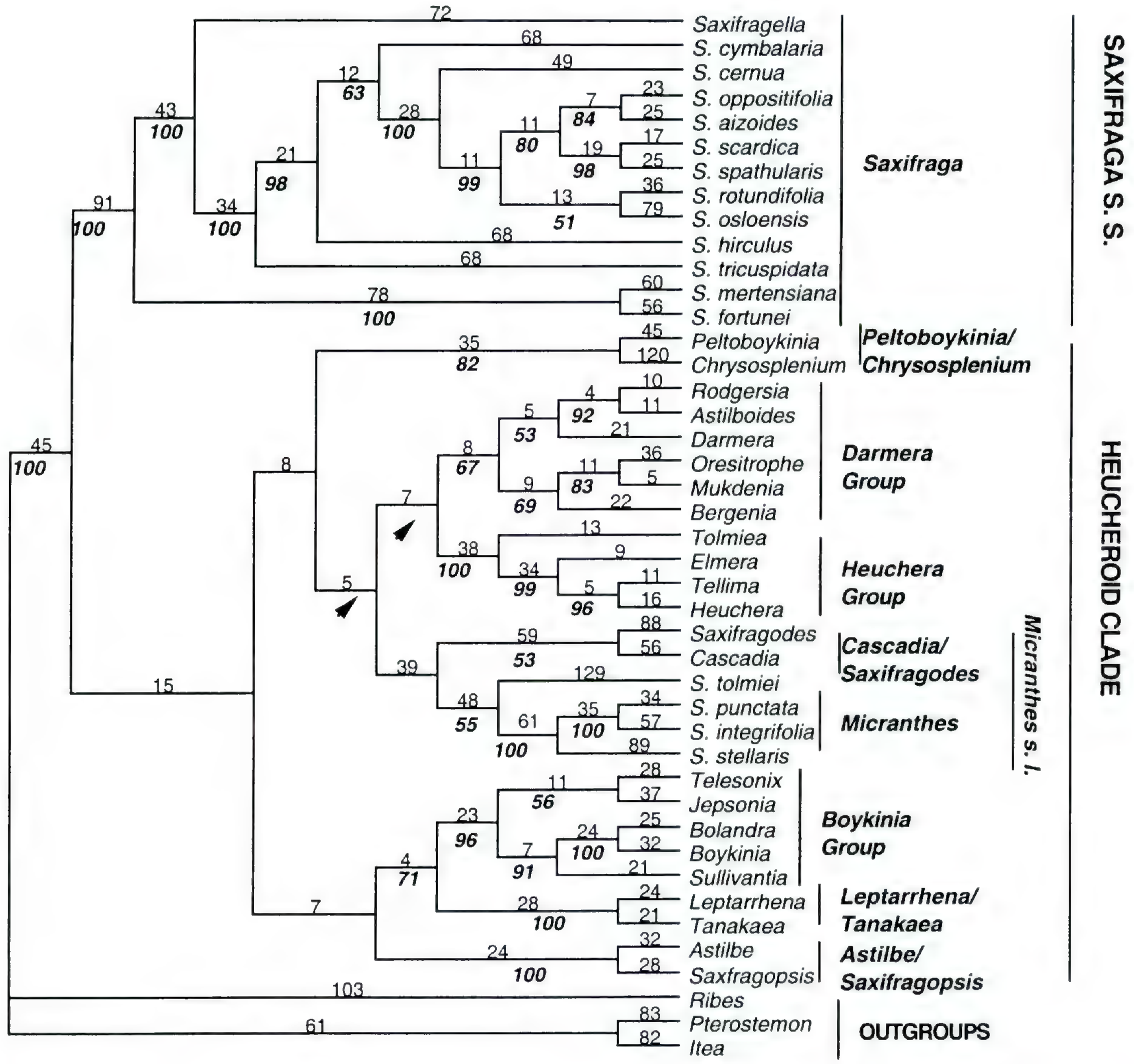


Figure 2. One of four shortest trees resulting from parsimony analysis of combined ITS and 26S rDNA sequence data; length = 3217, CI = 0.455, RI = 0.621. Percentages below branches are bootstrap values; numbers above branches are the number of base substitutions. Arrows indicate branches that collapse in the strict consensus of all shortest trees.

between *Saxifraga* s. str. and the heucheroids. *Saxifraga* s. str. is strongly supported by both nuclear and cpDNA data sets. However, the heucheroid clade receives bootstrap support greater than 50% only with the combined nuclear data set (61%). In addition, the same major clades and well-supported groups of genera are generally present with both. The following clades are present with bootstrap support greater than 50% in both the combined cpDNA and nuclear analyses: *Astilbe/Saxifragopsis* (100%/100%), *Boykinia* group (96%/100%), *Leptarrhena/Tanakaea* (100%/77%), *Peltoboykinia/Chrysosplenium* (78%/95%), *Cascadia/Saxifragodes* (58%/100%), *Micranthes* (100%/99%), *Micranthes* + *S. tolmiei* (58%/99%), *Heuchera* group (100%/100%), *Saxifraga* s. str. (100%/100%). The *Darmera* group is present in both analyses, but receives bootstrap support greater than 50% only with cpDNA.

Additional strong similarities between combined nuclear and combined cpDNA topologies are observed within those clades receiving bootstrap support greater than 50%. The relationships and clades receiving bootstrap support greater than 50% are nearly identical within *Saxifraga* s. str. and also within other subgroups, such as *Micranthes*, the *Heuchera* group, the *Darmera* group, and the *Boykinia* group.

The partition homogeneity test revealed similar levels of heterogeneity (values of 0.01 to 0.02) between all data set combinations, regardless of

whether they are nuclear or chloroplastic. This is not surprising given the sensitivity of this test (e.g., Sullivan, 1996; Johnson & Soltis, 1998). Thus, the heterogeneity between nuclear and cpDNA data sets appears no greater than that observed among cpDNA or between nuclear data sets. The one area of potential hard incongruence between the combined nuclear and combined cpDNA trees involves the relationships among the *Boykinia* group, *Astilbe/Saxifragopsis*, and *Leptarrhena/Tanakaea*. The combined cpDNA analysis places *Leptarrhena/Tanakaea* as sister to the *Boykinia* group with 70% support. *Astilbe/Saxifragopsis* are, in turn, the sister to the *Boykinia* group + *Leptarrhena/Tanakaea* clade, but without support greater than 50%. In contrast, the combined nuclear data sets suggest a sister relationship between the *Boykinia* group and *Astilbe/Saxifragopsis* with 87% bootstrap support; *Leptarrhena/Tanakaea* are their subsequent sister group (62%).

COMBINED cpDNA/NUCLEAR DATA SET

We explored both the impact of the six different ITS alignments and the inclusion and removal of five difficult to align regions of the ITS in phylogenetic analyses based on the combination of all six DNA regions (ITS + 26S rDNA + four cpDNA regions). For each ITS alignment we employed all of the difficult to align bp in one analysis; for a second six-gene analysis we eliminated all five hard-to-align ITS areas (see section on ITS sequences). Thus, we ran a total of 12 different parsimony analyses on combined cpDNA/nuclear data sets. We also performed MLE analyses on two of the combined data sets—the combined data set that employed the rough ITS alignment (total length = 6630 aligned bp) and the combined data set that employed what we considered the best or preferred ITS alignment (total length = 6676 aligned bp).

We found the impact of the different ITS alignments and the removal of base pairs to be relatively small. We observed similar variations in topology between alignments as obtained within alignments, with characters included or excluded. One difference clearly associated with alignment involves the placement of *Cascadia/Saxifragopsis*. The six gene data set that employed the rough ITS alignment places this pair as sister to the remainder of the heucheroid clade, whereas in the five other combined data sets (employing the remaining ITS alignments) *Cascadia/Saxifragopsis* form a *Micranthes* s.l. clade that also includes *Micranthes* and *Saxifraga tolmiei*. However, MLE analysis of the six gene data set that employed the rough ITS alignment yields the same topology obtained through parsimony searches employing the five other ITS alignments, suggesting that the parsimony placement of *Cascadia/Saxifragopsis* using this one alignment is spurious.

A topological difference that appears to be due to both alignment and the culling of hard to align bp involves *Leptarrhena* and *Tanakaea*. Typically, *Leptarrhena/Tanakaea* form a clade and appear as sister to *Boykina* group + *Astilbe/Saxifragopsis*; but in one alignment with base pairs removed they form a grade and appear as sister to the remaining taxa of the heucheroid clade. Other topological differences resulting from different alignments or the removal of bp are very minor and include the occasional placement of *Rodgersia* and *Astilboides* in a trichotomy with *Darmera*, rather than as sister taxa.

Considering the length of the shortest trees obtained via parsimony in all six combined cpDNA/nuclear data sets, both with all ITS sequence bp included and with five difficult to align ITS regions removed, one combined data set yields shorter trees than the remaining five, and it is discussed below (Fig. 3). This six gene data set involves the same ITS alignment that yielded the shortest trees when analyzed alone and when combined with 26S rDNA sequences. The longest trees (and lowest CI and RI values) were obtained for the six gene data set that employs the rough ITS alignment.

Parsimony analysis of all six DNA regions (using the preferred ITS alignment with no bp removed) resulted in four shortest trees of 5523 steps (CI = 0.538; RI = 0.654). MLE analysis resulted in a topology identical to that observed in one of the shortest trees obtained via parsimony. These trees indicate that the family comprises two major lineages which receive high internal support—*Saxifraga* s. str. (100%) and the heucheroid clade (80%). Many relationships within the *Saxifraga* s. str. clade receive strong internal support (greater than 90%). Relationships within the heucheroid clade are well resolved and exhibit greater than 50% support. The heucheroids comprises two weakly supported subclades: (1) the *Boykinia* group, *Astilbe/Saxifragopsis*, *Leptarrhena/Tanakaea* (58%); (2) *Heuchera* group, *Darmera* group, *Micranthes* s.l. (*Micranthes*, *Saxifraga tolmiei*, *Saxifragodes/Cascadia*), and *Peltoboykinia/Chrysosplenium* (75%). Within the latter clade, the *Heuchera* group appears as sister to a clade of the remaining taxa, which form a clade with low (75%) support. The *Darmera* group is sister to another weakly supported clade (66%) that consists of *Micranthes* s.l. as sister to *Peltoboykinia/Chrysosplenium*.

In most cases, support for individual clades in-

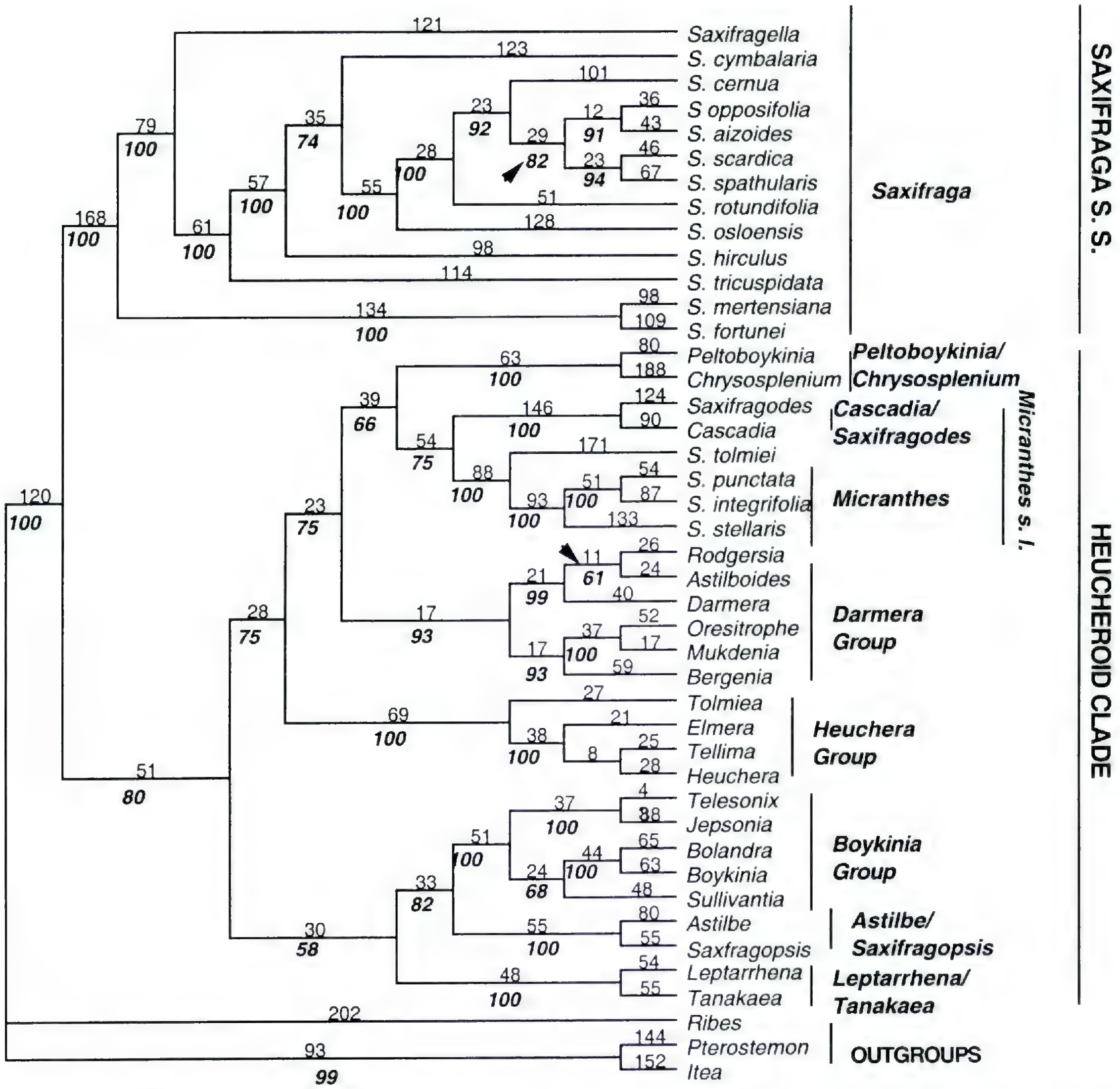


Figure 3. One of four shortest trees resulting from parsimony analysis of combined cpDNA (*rbc*L, *mat*K, *trn*L-*trn*F, *psb*A-*trn*H) and nuclear data sets (ITS and 26S rDNA); length = 5523, CI = 0.538, RI = 0.654. Percentages below branches are bootstrap values; numbers above branches are the number of base substitutions. Arrows indicate branches that collapse in the strict consensus of all shortest trees.

creased in the analyses of all six regions compared to both the combined cpDNA and nuclear trees (compare Fig. 3 with Figs. 1 and 2). Support for many tip clades within Saxifragaceae increases to 100% in the six DNA region data set (*Astilbe/Saxifragopsis; Boykinia* group; *Leptarrhena/Tanakaea; Heuchera* group; *Peltoboykinia/Chrysosplenium*; *Micranthes* s.l., *Micranthes*; *Cascadia/Saxifragodes*; *Saxifraga* s. str., including *Saxifragella*). Support for the *Darmera* group increases to 91% and that for the heucheroid clade increases to 89%. However, because of conflict between the cpDNA and nuclear trees, support for the sister group relationship between the *Boykinia* group and *Astilbe/Saxifragopsis* decreases from 87% in the combined nuclear regions to 73% for all six genes. *Leptarrhena/Tanakaea* remain as sister to *Boykinia* group *Astilbe/Saxifragopsis*, but with weak support (58%).

Discussion

Previous phylogenetic analyses of Saxifragaceae have employed separate (Soltis et al., 1993) and combined (Soltis et al., 1996a) data sets of *mat*K and *rbc*L sequences, as well as ITS sequences (Johnson & Soltis, 1998). Those analyses provided support for many groups of genera within the family (i.e., *Boykinia*, *Heuchera*, and *Darmera* groups,

Saxifraga s. str., *Micranthes*, *Astilbe/Saxifragopsis*, *Leptarrhena/Tanakaea*, *Chrysosplenium/Peltoboykinia*). However, relationships among these tip clades were unclear. In an effort to clarify deep-level relationships within the family we sequenced two additional cpDNA regions (*trn*L-*trn*F and *psb*A-*trn*H) and most of the 26S rDNA expansion segments; we compiled a final combined data set for six genes/DNA regions of 6676 aligned bases (5601 bp were ultimately employed). We also added *Saxifragella* and *Saxifragodes*, two monotypic genera from the southernmost portion of South America for which material had not been previously available.

GENERAL CONSIDERATIONS

The addition of sequences from two chloroplast spacer regions (*trn*L-*trn*F and *psb*A-*trn*H) supported the monophyly of many of the well-defined groups earlier revealed by *mat*K and *rbc*L sequences (Soltis et al., 1993, 1996a). Significantly, *trn*L-*trn*H sequence data provided a topology, as well as resolution and support, comparable to what was obtained with *mat*K and *rbc*L. The *psb*A-*trn*H spacer was difficult to align in Saxifragaceae; the small number of bp we were able to employ provided low resolution and support. In retrospect, sequencing of an additional chloroplast gene or genes such as *atp*B and/or *ndh*F may have been more valuable than the use of the *psb*A-*trn*H spacer region.

Nuclear gene sequence data derived from ITS and 26S rDNA expansion segments provided additional support for the same tip clades revealed by cpDNA sequences. Again, however, deep-level relationships remained poorly resolved and supported. These data further demonstrate the phylogenetic potential of sequences derived from 26S rDNA expansion segments (e.g., Kuzoff et al., 1998; Ashworth, 2000). We experimented extensively with different ITS alignments and with the exclusion of difficult to align regions. Comparisons of trees obtained using different ITS alignments and removal of hard-to-align regions reveal only minor topological differences, with no impact on well-supported clades. These results reinforce the similar findings of Soltis et al. (1996b) and Wheeler et al. (1995). For example, Soltis et al. (1996b) experimented with 18 different ITS alignments, as well as with culled data sets for the *Boykinia* group and found that alignment and removal of tenuously aligned bp had little impact. The data suggest that extensive experimentation with ITS alignments may not be necessary; different alignments, even those considered relatively rough, may have little or no impact on well-supported groups. In some cases, the removal of hard-to-align regions does result in less resolution.

We also employed MLE analyses, comparing the results to those obtained with parsimony. In virtually all instances the MLE topology obtained is identical to one of the shortest trees obtained via parsimony. The only exception involved the analysis of ITS sequences. Inclusion of *Ribes* (characterized by a long branch) resulted in shortest trees for several different alignments in which *Saxifragodes/Cascadia* (also with long branches) appear as sister to the remaining members of Saxifragaceae. When *Ribes* was removed, parsimony analyses place *Saxifragodes/Cascadia* in the expected position, near *Micranthes*. MLE analyses that employ *Ribes* as an outgroup also place *Saxifragodes/Cascadia* near *Micranthes*. Thus, MLE analyses seem to resolve successfully a relationship in which parsimony analyses sometimes fail.

DISCORDANCE BETWEEN cpDNA AND NUCLEAR TREES

Comparison of two combined nuclear regions (ITS and 26S rDNA) and four combined cpDNA regions (*mat*K, *rbc*L, *trn*L-*trn*F, *psb*A-*trn*H) reveal very similar nuclear- and cpDNA-based topologies, with numerous well-supported clades (compare Figs. 1 and 2). Despite the strong overall similarities between the cpDNA and nuclear topologies, the trees do differ in the relationships among the *Boykinia* group, *Astilbe/Saxifragopsis*, and *Tanakaea/Leptarrhena*. Although nuclear and cpDNA sets agree that the *Boykinia* group, *Astilbe/Saxifragopsis* and *Tanakaea/Leptarrhena* are closely related, the combined cpDNA analysis places *Leptarrhena/Tanakaea* as sister to the *Boykinia* group with 70% support; *Astilbe/Saxifragopsis* are then their sister, but without support greater than 50%. In contrast, the combined nuclear data suggest a strongly supported (87%) sister group relationship between the *Boykinia* group and *Astilbe/Saxifragopsis; Leptarrhena/Tanakaea* are their subsequent sister group (62%). This difference in the sister to the *Boykinia* group (*Astilbe/Saxifragopsis* versus *Leptarrhena/Tanakaea*) appears to represent a case of hard incongruence given that the nuclear and cpDNA placements each receive bootstrap support greater than or equal to 70% (Mason-Gamer & Kellogg, 1996; Seelanen et al., 1997). The difference between cpDNA and nuclear placements appears to be robust.

Several phenomena may lead to conflicting phylogenetic hypotheses and include both organism-level and genome-level processes (reviewed in

Wendel & Doyle, 1998). The reasons for the incongruence in Saxifragaceae are unclear, however. One obvious possibility is an ancient episode of hybridization/introgression, a process that is well known within some groups of Saxifragaceae (e.g., Soltis et al., 1991a, 1991b; Soltis & Kuzoff, 1995; Soltis et al., 1996b; Kuzoff et al., 1999). The *Boykinia* group, *Leptarrhena/Tanakaea,* and *Saxifragopsis/Astilboides* are primarily diploid lineages that share $2n = 14$. A putative hybridization/introgression event would have had to involve taxa representing the *Boykinia* group and the *Leptarrhena/Tanakaea* clade. Subsequent chloroplast capture by a member of the latter clade resulted in a sister group cpDNA relationship between the *Boykinia* group and *Leptarrhena/Tanakaea.* Nuclear markers would then be more likely to reflect the actual organismal relationship, with *Astilbe/Saxifragopsis* the sister to the *Boykinia* group. Following this scenario, the putative hybridization and chloroplast capture event would have been ancient, likely occurring during the early diversification of these clades. Hybridization/chloroplast capture has been frequently documented within Saxifragaceae, not only within genera such as *Heuchera,* but also between species of distinct genera of the *Heuchera* group such as *Heuchera* and *Tiarella* and *Tellima* and *Mitella* (Soltis et al., 1991a; Soltis & Kuzoff, 1995). In the latter case, the species in which chloroplast capture was documented presently do not hybridize (Soltis et al., 1991b). Hybridization and chloroplast capture also appear to have occurred in the early diversification of the *Boykinia* group (Soltis et al., 1996b).

RESOLVING DEEP-LEVEL RELATIONSHIPS

Earlier analyses of relationships within Saxifragaceae using one or two combined genes generally provided strong support for tip clades, but did not resolve deep-level relationships (Soltis et al., 1993, 1996a). Phylogenetic trees of this type have been encountered often, and at a number of different levels (e.g., Baldwin et al., 1998; Olmstead et al., 1993; Hershkovitz & Zimmer, 1997; Mort et al., in press; Jansen & Kim, 1996) and are usually interpreted as instances of rapid radiation, although there are alternative causes of poor resolution at deep levels (reviewed in Soltis & Soltis, 1995).

The difficulty in resolving short, deep branches has recently been considered by several investigators (e.g., Donoghue & Sanderson, 1992; Baldwin et al., 1998; Flook et al., 1999; Fishbein et al., 2001). Characters should evolve rapidly during the period of "rapid radiation" yet evolve relatively slowly subsequent to the radiation to the present (Donoghue & Sanderson, 1992). Alternatively, sufficient genes and DNA regions with moderate rates of evolution must be sequenced that by chance sufficient base changes are detected on those short nodes to permit resolution. The challenge these short branches pose is illustrated by several recent studies. For example, Fishbein et al. (2001) used approximately 9000 bp of sequence data from five genes in an effort to resolve deep branches in Saxifragales and still obtained poor resolution and support at that level. They estimated that an extraordinary effort ($\sim 10^7$ bp) might ultimately be needed to resolve this problem.

Whatever the underlying cause for low resolution among lineages in Saxifragacae (rapid radiation or some other factor), our study joins other recent analyses in demonstrating the value of adding characters to resolve deep-level relationships in problematic groups. Compared to a combined *mat*K/*rbc*L data set (~ 2500 bp) for Saxifragaceae (Soltis et al., 1996a), our analyses of six combined nuclear and cpDNA data sets (6676 bp) not only provide increased support for the tip clades, but also reveal increased resolution and internal support for deep-level relationships within the Saxifragaceae.

Other investigators have similarly met with success in tackling problematic deep level relationships by adding characters. A recent example at the family level involves the Poaceae; relationships were eventually resolved through analyses of eight combined data sets (Grass Phylogeny Working Group, 2000). Another success story in resolving deep level relationships involves the identification of the first-branching angiosperms (Amborellaceae, Nymphaeaceae, Illiciales, Austrobaileyaceae, Trimeniaceae), a problem once considered particularly enigmatic and perhaps intractable (reviewed in Chase & Cox, 1998; Kuzoff & Gasser, 2000). However, the combination of three genes (approximately 4700 bp) resolved these first-branches with bootstrap support greater than 50% (Soltis et al., 1999); the use of five genes (8733 aligned bp) did so with bootstrap support greater than 90% (Qiu et al., 1999; see also Parkinson et al., 1999; Graham & Olmstead, 2000). However, after these first branches, relationships among the remaining non-eudicot clades (Laurales, Magnoliales, Winterales, Chloranthales, Ceratophyllaceae, monocots) still remained unclear, with bootstrap support greater than 50%. Significantly, the use of six genes (about 12,000 bp of sequence data) greatly improved resolution and provided strong internal support for most relationships (Zanis et al., in prep.). These results emphasize that the addition of still more

sequence data is often worthwhile in the effort to resolve problematic deep-level relationships.

PHYLOGENETIC RELATIONSHIPS

With six DNA regions, most relationships within the Saxifragaceae are resolved and receive support greater than 50% (Fig. 3). Two major clades are revealed, *Saxifraga* s. str. (100%) and the heucheroid clade (80%); this is the first analysis to provide strong support for these two major clades. Relationships within *Saxifraga* s. str. receive strong internal support (greater than 90%); however, previous analyses based only on one or two genes also resolved relationships with moderate to strong support within this clade (Soltis et al., 1996a). The most noteworthy results regarding increased resolution and support of relationships involve the heucheroid clade. Although previous studies provided support for most of the tip clades within the heucheroid clade, deep-level relationships were uncertain with no relationships receiving support greater than 50%. With six DNA regions, relationships within the heucheroid clade now appear well resolved and exhibit support greater than 50% (Fig. 3).

GENERAL PHYTOGEOGRAPHIC AND MORPHOLOGICAL CONSIDERATIONS

The major split revealed in Saxifragaceae between *Saxifraga* s. str. and the heucheroid clade is accompanied by general biogeographical differences. Our data suggest that the family diverged early into a largely arctic-alpine lineage (*Saxifraga* s. str.) and a primarily temperate lineage (heucheroid clade). Of the 13 sections that would remain in *Saxifraga* s. str. (following removal of sections *Micranthes* and *Merkianae* to form the distinct genus *Micranthes*), most are either montane or arctic in distribution (Webb & Gornall, 1989). *Saxifragella* (now part of *Saxifraga*) also fits this pattern, being confined to Tierra del Fuego. *Saxifraga* sect. *Irregulares* deviates from this pattern, with some species occurring in woodlands in Asia. Centers of distribution for *Saxifraga* would include the mountains of Europe and Asia.

In contrast, the heucheroid clade is largely north-temperate in distribution with a major center of diversification in western North America, the area in which representatives of most genera of Saxifragaceae can be found (e.g., *Bensoniella, Bolandra, Cascadia, Darmera, Elmera, Heuchera, Jepsonia, Leptarrhena, Lithophragma, Micranthes, Saxifragopsis, Suksdorfia, Telesonix, Tellima,* and *Tolmiea*). A second area of diversity for the heucheroid clade is temperate areas of eastern Asia (*Astilbe, Rodgersia, Astilboides, Tanakaea, Oresitrophe, Peltoboykinia, Mukdenia, Bergenia, Chrysosplenium, Micranthes*). Within the heucheroid clade, a secondary radiation into arctic/alpine areas is observed in some *Micranthes*.

The major split in Saxifragaceae between *Saxifraga* and the heucheroid clade is also accompanied by general differences in patterns of morphological evolution (patterns of evolution will be explored in more detail on a character by character basis via mapping experiments in a subsequent paper). *Saxifraga* s. str. has a relatively uniform floral morphology (actinomorphic, with zygomorphy present in one section, *Irregulares*; 5 sepals, 5 petals, 10 stamens, 2 carpels), whereas the heucheroid clade encompasses much more floral variation. In the *Heuchera* group, actinomorphic flowers predominate, but there are some zygomorphic forms (*Tolmiea, Bensoniella,* some species of *Heuchera*). In addition, virtually all of the familial variation in number of sepals, petals, stamens, and carpels occurs within this subclade. Sepal number ranges from none to ten and petals from none to six. In contrast to *Saxifraga*, petal morphology is also highly variable, with spatulate, linear, lobed, and highly dissected petals all present. There is also stamen variation, with stamen number ranging from 3 to 10, with diplostemony, haplostemony, and obhaplostemony present. Whereas carpel number in *Saxifraga* and the heucheroid clade is standardly two, there is some variation in this feature in the latter clade, with *Lithophragma* possessing three carpels.

SOUTH AMERICAN DISJUNCTS

Our analyses also provide insights into the taxa occurring disjunctly in South America. Several monotypic genera (*Hieronymusia, Saxifragella,* and *Saxifragodes*) occur in southern regions of South America, well isolated geographically from other members of the family. In addition, *Saxifraga* s. str. and *Chrysosplenium* also possess several species restricted to South America. Molecular data (Soltis et al., 2001) recently provided important insights into the disjunction involving species of *Chrysosplenium* that may be generally applicable to the other South American disjuncts.

Chrysosplenium consists of 57 species widely distributed in temperate and arctic regions of the Northern Hemisphere, with two species restricted to the southern part of South America. Molecular phylogenetic analyses not only suggest that the genus arose in eastern Asia, but also indicate that the

Table 4. Tribes of Saxifragoideae following Schulze-Menz (1964), which are essentially equivalent to Engler's (1930) subtribes of Saxifrageae. Generic names and boundaries in Saxifrageae have been updated.

Astilbeae	Leptarrheneae	Saxifrageae	
Astilbe	*Leptarrhena*[a]	*Bensoniella*[a]	*Mukdenia*[a]
Rodgersia	*Tanakaea*[a]	*Conimitella*[a]	*Telesonix*[b]
Astilboides[a]		*Elmera*[a]	*Hieronymusia*[a,c]
		Heuchera	*Bolandra*
		Lithophragma	*Boykinia*
		Mitella	*Suksdorfia*
		Tolmiea[a]	*Peltoboykinia*[a]
		Tellima[a]	*Saxifragella*[a]
		Jepsonia	*Zahlbrucknera*[a,d]
		Oresitrophe[a]	*Chrysosplenium*
		Bergenia	*Saxifraga*
		Darmera[a]	

[a] Designates a monotypic genus.
[b] Submerged into *Boykinia* by Gornall and Bohm (1985).
[c] Submerged into *Suksdorfia* by Gornall and Bohm (1985).
[d] Submerged into *Saxifraga*; see Webb and Gornall (1989) and Soltis et al. (1996a).

closest relatives of the South American species are from eastern Asia (Soltis et al., 2001). Sequence divergence values suggest that the disjunction is not recent in origin. Taken together, the data suggest ancient long-distance dispersal. A similar argument was posed by Schnabel and Wendel (1998) for a comparable disjunction in the genus *Gleditsia* (Fabaceae). Alternatively, this disjunction could represent remnants of a once more continuous geographic distribution, with subsequent extinction in much of the intervening area. Interrupted migration of plants between North and South America was possible via the West Indies as early as the Oligocene (which does not seem applicable here), and direct migration between North and South America became feasible 5.7 million years ago when the union of North and South America occurred (Raven & Axelrod, 1974). However, the absence of members of this alliance in the large intervening area between Asia and southern South America argues against this scenario. Suitable habitats seem to be present given that species from other subclades are found throughout the west extending south to California. Thus, the available data would seem to favor ancient long-distance dispersal.

The present study suggests that there may be a general pattern and common explanation regarding the South American disjuncts in Saxifagaceae. That is, ancient long-distance dispersal, from either eastern Asia or western North America, may be a likely explanation for most, if not all, of the other saxifragalean disjuncts in South America.

Saxifragella, confined to Tierra del Fuego, appears as an early-branching member of the large *Saxifraga* s. str. clade. A small clade of *S. mertensiana* and *S. fortunei* (representing *Saxifraga* sect. *Irregulares*) is the sister group to the remainder of *Saxifraga* s. str., with *Saxifragella* following this clade as the next branching member of the genus. Most members of *Saxifraga* s. str. occur in Europe, where 11 of the 13 sections are located (the diversity of the genus *Saxifraga* s. str. is well represented in our topology; see also Soltis et al., 1996a). However, *Saxifraga mertensiana* occurs in northwestern North America extending into Alaska, and *Saxifraga* sect. *Irregulares* (with *S. fortunei*) is restricted to eastern Asia. Thus, the first branching members of *Saxifraga* s. str. are all from the Pacific Rim. Based on the six gene topology, *Saxifragella* is also well differentiated in its sequences from other species of *Saxifraga*. These phylogenetic data suggest that *Saxifragella* is a relictual taxon, potentially representing a remnant of the early radiation and diversification of *Saxifraga* s. str. that survived at the southern tip of South America. These results are also in agreement with an ancient long-distance dispersal event from either eastern Asia or the Pacific Northwest of North America to the southern tip of South America.

An intriguing disjunction involves two enigmatic, monotypic genera, *Cascadia* and *Saxifragodes*, which our analyses reveal to be sister taxa. These two taxa are narrow endemics, confined to western Washington and Oregon and Tierra del Fuego, respectively. Until this study, the affinities of these genera were obscure, although a close relationship, or actual inclusion in, *Saxifraga* had been proposed for both (Engler & Irmscher, 1916–1919). Although

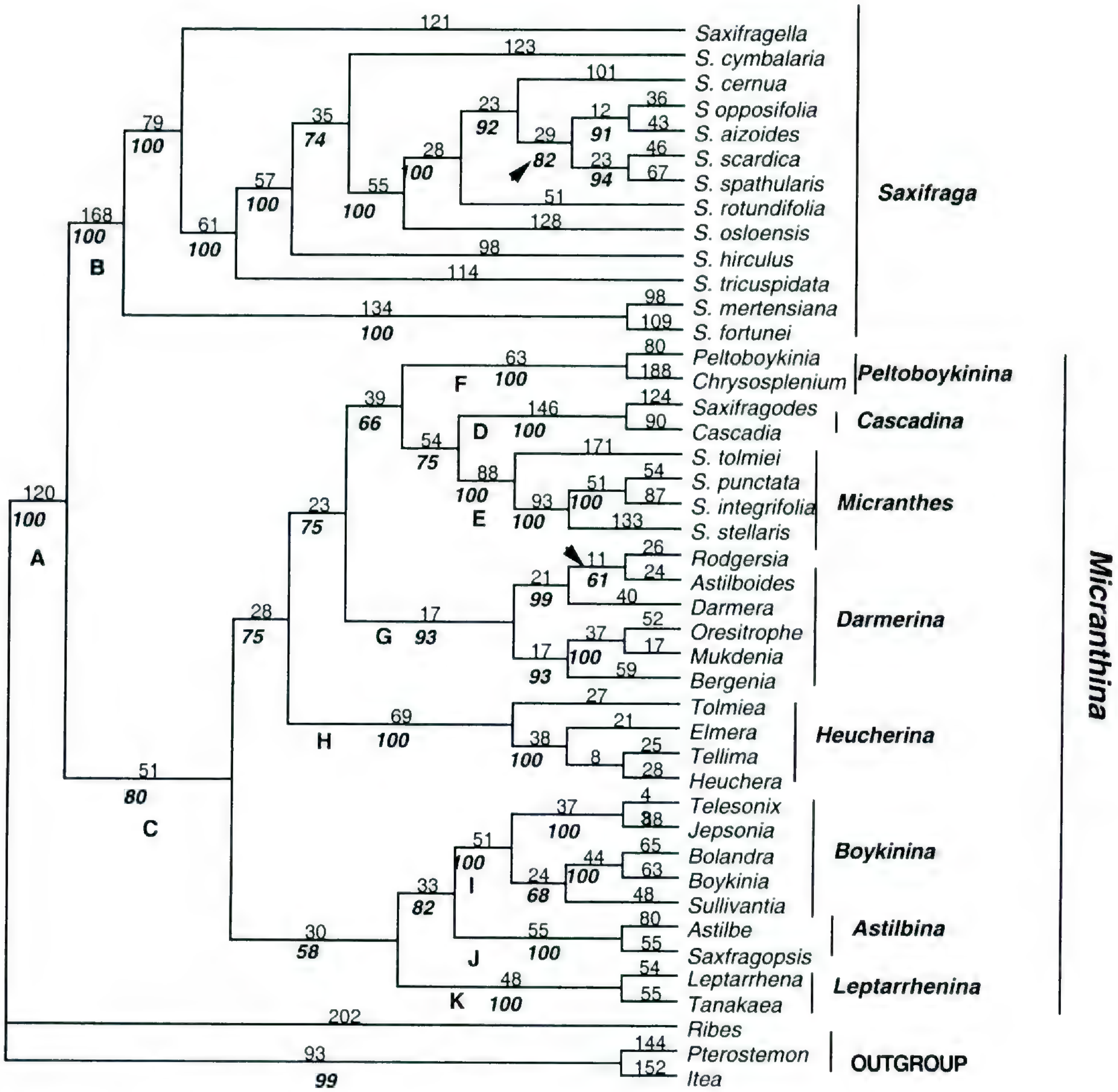


Figure 4. Same tree as in Figure 3, modified to show names used in reclassification of the family Saxifragaceae (see Table 5 and Appendix). Letters correspond to clade names given in Table 5 and the Appendix.

morphologically quite distinctive, both genera share similar habitats (wet, moss-covered soils and rocks), as well as a similar creeping habit unusual in the family. Although well supported as sister taxa, the genera are highly diverged based on all of the gene sequences analyzed. The data again suggest a possible ancient long-distance dispersal event, in this case from the Pacific Northwest of North America to southern South America.

There are also several species of *Saxifraga* s. str. in southern South America; these occur in the Andes, some as far north as Colombia (*S. magellanica*, *S. adenoides*, *S. pavonii*, and *S. boussingaultii*; Webb & Gornall, 1989). These species are all members of *Saxifraga* sect. *Saxifraga*; a large section that occurs at high elevations and high latitudes throughout the Northern Hemisphere. The closest relatives of the South American species within section *Saxifraga* are unknown, but the data are certainly suggestive of still additional long-distance dispersal events to South America.

Savile (1953, 1975) provided hypotheses for seed dispersal for members of Saxifragaceae. Species of *Mitella* and *Chrysosplenium* have fruits that he termed "splash cups" with the seeds splashed out by drops of water falling from the canopy. Species of *Chrysosplenium* typically occur on wet forest floors, under dense forest canopies, all of which would make long-distance dispersal on a large geographic scale difficult. The structure of the fruits and infructescences of other species are hypothesized to function to release seeds via vibration in

response to the wind. Although none of these proposed seed dispersal mechanisms appears particularly well suited for long-distance seed dispersal, seeds of all Saxifragaceae are small, so long-distance dispersal of seeds is a possibility.

Taxonomic Revision

We have been conducting systematic investigations of Saxifragaceae for two decades. Flavonoid and karyotypic data suggested the presence of natural groups of genera, referred to informally as the *Heuchera* and *Boykinia* groups (e.g., Gornall & Bohm, 1985; Soltis, 1980), that were at odds with the traditional classification of the family (Table 4). DNA-based studies later confirmed the monophyly of these groups (Soltis et al., 1991a, 1996b), indicated that *Saxifraga* was polyphyletic (Soltis et al., 1996a), and also suggested the presence of other clades (e.g., *Darmera* group, *Saxifragopsis/Astilbe*, *Chrysosplenium/Peltoboykinia*) that did not agree with tribal or subtribal groupings (Engler, 1930; Schulze-Menz, 1964). Although these studies indicated clearly that Saxifragaceae required taxonomic revision, deep-level relationships remained unclear and material of several enigmatic monotypic genera could not be procured at that time.

This study not only resolves deep-level relationships and provides support for most clades within Saxifragaceae, but also places the monotypic genera *Saxifragella* and *Saxifragodes*. The well-supported major clades, as well as the tip clades show little agreement with past taxonomic treatments of the family. Engler (1930) and Schulze-Menz (1964) proposed similar treatments of subfamily Saxifragoideae (one of 15 or 17 subfamilies of a broadly defined Saxifragaceae s.l.), which is equivalent to our circumscription of Saxifragaceae. Engler recognized three subtribes, which were later elevated to tribes by Schulze-Menz (see Table 4). Tribes Astilbeae and Saxifrageae do not correspond to any clades revealed by our DNA analyses. Only tribe Leptarrheneae corresponds to a clade, the two genera (*Leptarrhena* and *Tanakaea*) sharing an unusual anther morphology found nowhere else in the family. Klopfer (1973) divided the family into two informal groups, one centered on *Heuchera* (corresponding to the *Heuchera* group) and a second group comprising all other genera.

Because genera of Saxifragaceae typically are small, for most we have included all, or nearly all, species in our analyses. The large genera (*Saxifraga* s. str., *Micranthes*, *Heuchera*, and *Chrysosplenium*) have also been very well sampled (Soltis et al., 1991a, 1996a; Mort & Soltis, 1999). As noted, only two monotypic genera, both rare and local in occurrence, have not been included in molecular analyses of Saxifragaceae, *Saniculiphyllum* and *Hieronymusia*. The affinities of *Saniculiphyllum*, which has only been collected once (Wu & Ku, 1992), are obscure. However, because of its distinctive chemistry and morphology, *Hieronymusia* is thought to be closely related to *Suksdorfia* (Gornall & Bohm, 1985). The relationships of the two species of *Suksdorfia* were established in earlier molecular phylogenetic analyses of the *Boykinia* group (Soltis et al., 1996b). Given our firm understanding of relationships and thorough sampling of Saxifragaceae, it seems timely to propose a revised taxonomy for the family.

There has been considerable interest (e.g., Cantino et al., 1997; Cantino, 1998; Kron, 1997; Hibbett & Donoghue, 1998) and contention (e.g., Stuessy, 2000) regarding phylogenetic systems of classification. The advantages of a phylogenetic system have been thoroughly reviewed. We only add that a phylogenetic system, in this case, is a practical and useful one. Only one formal taxonomic name (Leptarrheneae) corresponds to a clade detected in our recent phylogenetic analyses. Thus, most clades in Saxifragaceae have only been known by informal names; some (*Heuchera* and *Boykinia* groups) for nearly 20 years.

We present here (Fig. 4; Table 5; Appendix) a revised classification for Saxifragaceae that follows a phylogenetic system of nomenclature, as proposed by De Queiroz and Gauthier (1990, 1992). The names presented (Fig. 4; Table 5; Appendix) are

Table 5. Phylogenetic classification of Saxifragaceae following the method of de Queiroz and Gauthier (1992). This is a rank-free classification. Clades, except those classified as genera in previous treatments (e.g., *Micranthes*, *Saxifraga*), are assigned names with the standard ending "ina." Letters given to clades correspond to those in Figure 4 and the Appendix.

Saxifragaceae	(clade A)
Saxifraga	(clade B)
Micranthina	(clade C)
Cascadina	(clade D)
Micranthes	(clade E)
Peltoboykinina	(clade F)
Darmerina	(clade G)
Heucherina	(clade H)
Boykinina	(clade I)
Astilbina	(clade J)
Leptarrhenina	(clade K)
Saniculiphyllum[a]	

[a] The phylogenetic relationships of this genus remain obscure.

not correspondent to nomenclatural equivalents under the ICBN (Greuter et al., 2000), but are applied following the Phylocode. We base our classification on the topology presented here and on the results of phylogenetic analyses focused on subclades within Saxifragaceae, such as the *Heuchera* (Soltis et al., 1991a; Soltis & Kuzoff, 1995) and *Boykinia* groups (Soltis et al., 1996b), *Micranthes* (Mort & Soltis, 1999), and *Saxifraga* s. str. (Soltis et al., 1996a).

Literature Cited

Ashworth, V. E. T. M. 2000. Phylogenetic relationships in Phoradendreae (Viscaceae) inferred from three regions of the nuclear ribosomal cistron. I. Major lineages and paraphyly of *Phoradendron*. Syst. Bot. 25: 349–370.

Baldwin, B. G., D. J. Crawford, J. Francisco-Ortega, S.-C. Kim, T. Sang & T. F. Stuessy. 1998. Molecular phylogenetic insights on the origin and evolution of oceanic island plants. Pp. 110–141 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II. Kluwer, Boston.

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford & P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. Syst. Biol. 42: 384–397.

Cantino, P. D. 1998. Binomials, hyphenated uninomials, and phylogenetic nomenclature. Taxon 47: 425–429.

———, R. G. Olmstead & S. J. Wagstaff. 1997. A comparison of phylogenetic nomenclature with the current system: A botanical case study. Syst. Biol. 46: 313–331.

Chase, M. W. & A. V. Cox. 1998. Gene sequences, collaboration, and analysis of large data sets. Austral. Syst. Bot. 11: 215–229.

Donoghue, M. J. & M. J. Sanderson. 1992. The suitability of molecular and morphological evidence in reconstructing plant phylogeny. Pp. 340–368 *in* P. S. Soltis, D. E. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants. Chapman and Hall, New York.

Engler, A. 1930. Saxifragaceae. *In* A. Engler & K. Prantl (editors), Die natürlichen Pflanzenfamilien, 2nd ed., Vol. 18a, 74–226. Engelmann, Leipzig.

——— & E. Irmsher. 1916–1919. *Saxifraga*. *In* A. Engler (editor), Das Pflanzenreich IV. 117: 1–709. Engelmann, Leipzig.

Farris, J. S., M. Källersjö, A. G. Kluge & C. Bult. 1995. Testing significance of incongruence. Cladistics 10: 315–319.

Fishbein, M., C. Hibsch-Jetter, D. E. Soltis & L. Hufford. 2001. Phylogeny of Saxifragales (Angiosperms: Eudicots): Analysis of a rapid, ancient radiation. Syst. Biol. 50.

Flook, P. K., S. Klee & C. H. F. Rowell. 1999. Combined molecular phylogenetic analysis of the Orthoptera (Arthropoda, Insecta) and implications for their higher systematics. Syst. Biol. 48: 233–253.

Gielly, L. & P. Taberlet. 1994. The use of chloroplast DNA to resolve plant phylogenies: Non-coding versus *rbc*L sequences. Molec. Biol. Evol. 11: 769–777.

——— & ———. 1996. A phylogeny of the European gentians inferred from chloroplast *trn*L (UAA) intron sequences. Bot. J. Linn. Soc. 120: 57–75.

Gornall, R. J. 1987. An outline of a revised classification of *Saxifraga* L. Bot. J. Linn. Soc. 7: 233–238.

——— & B. A. Bohm. 1985. A monograph of *Boykinia*, *Peltoboykinia*, *Bolandra*, and *Suksdorfia* (Saxifragaceae). Bot. J. Linn. Soc. 90: 1–71.

Graham, S. W. & R. G. Olmstead. 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. Amer. J. Bot. 87: 1712–1730.

Grass Phylogeny Working Group. 2000. A phylogeny of the grass family (Poaceae), as inferred from eight character sets. Pp. 3–7 *in* S. W. L. Jacobs & J. E. Everett (editors), Grasses—Systematics and Evolution, Vol. 2 of Proceedings of the Second International Conference on the Comparative Biology of the Monocots. CSIRO Publishing, Victoria, Australia.

Graybeal, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47: 9–17.

Greuter, W., J. McNeill, F. R. Barrie, H. M. Burdet, V. Demoulin, T. S. Filgueiras, D. H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland & D. L. Hawksworth (Editors). 2000. International Code of Botanical Nomenclature (Saint Louis Code). Regnum Veg. 138.

Gu, X., Y.-X. Fu & W.-H. Li. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Molec. Biol. Evol. 12: 546–557.

Hara, H. 1957. Synopsis of the genus *Chrysosplenium* L. J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 7: 1–90.

Haworth, A. H. 1812. Synopsis plantarum succulentarum. viii + 334 pp. London.

Hershkovitz, M. A. & E. A. Zimmer. 1997. On the evolutionary origins of the cacti. Taxon 46: 217–232.

Hibbett, D. S. & M. J. Donoghue. 1998. Integrating phylogenetic analysis and classification in fungi. Mycologia 90: 347–356.

Hillis, D. M. 1996. Inferring complex phylogenies. Nature 383: 130.

Huelsenbeck, J. P., J. J. Bull & C. W. Cunningham. 1996. Combining data in phylogenetic analysis. TREE 11: 152–158.

Jansen, R. K. & K-J. Kim. 1996. Implications of chloroplast DNA data for the classification and phylogeny of the Asteraceae. Pp. 317–339 *in* D. J. N. Hind & H. Beentje (editors), Compositae: Systematics. Proceedings of the International Compositae Conference, Kew, Vol. 1. Royal Botanical Gardens, Kew.

Johnson, L. A. & D. E. Soltis. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxifragaceae sensu stricto. Syst. Bot. 19: 143–156.

——— & ———. 1995. Phylogenetic inference using *mat*K sequences. Ann. Missouri Bot. Gard. 82: 149–175.

——— & ———. 1998. Assessing congruence: Empirical examples from molecular data. Pp. 297–348 *in* D. Soltis, P. Soltis & J. Doyle (editors), Molecular Systematics of Plants II. Kluwer, Boston.

Klopfer, K. 1973. Florale Morphogenese und Taxonomie der Saxifragaceae sensu lato. Feddes Repert. Beih. 84: 475–516.

Kron, K. A. 1997. Exploring alternative systems of classification. Aliso 15: 105–112.

Kuzoff, R. K. 1998. The Diversification of Ovary Architecture in the *Heuchera* group of Genera (Saxifragaceae). Ph.D. Thesis, Washington State University, Pullman.

——— & C. S. Gasser. 2000. Rapid progress in flowering plant phylogeny. Trends Pl. Sci. 5: 330–336.

———, J. A. Sweere, D. E. Soltis, P. S. Soltis & E. A. Zimmer. 1998. The phylogenetic potential of entire 26S rDNA sequences in plants. Molec. Biol. Evol. 15: 251–263.

———, D. E. Soltis, L. Hufford & P. E. Soltis. 1999. Phylogenetic relationships within *Lithophragma* (Saxifragaceae): Hybridization, allopolyploidy, and ovary diversification. Syst. Bot. 24: 598–615.

———, L. Hufford & D. E. Soltis. 2001. Structural homology and developmental transformations associated with ovary diversification in *Lithophragma* (Saxifragaceae). Amer. J. Bot. 88: 196–205.

Lledó, M. D., M. B. Crespo, K. M. Cameron, M. F. Fay & M. W. Chase. 1998. Systematics of Plumbaginaceae based upon cladistic analysis of *rbc*L sequence data. Syst. Bot. 23: 21–29.

Maddison, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Syst. Zool. 40: 315–328.

Mason-Gamer, R. J. & E. A. Kellogg. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. 45: 522–543.

Miyamoto, M. M. & W. M. Fitch. 1995. Testing species phylogenies and phylogenetic methods with congruence. Syst. Biol. 44: 64–76.

Mort, M. E. & D. E. Soltis. 1999. Phylogenetic relationships and the evolution of ovary position in *Saxifraga* section *Micranthes*. Syst. Bot. 24: 139–147.

———, ———, P. S. Soltis, J. Francisco-Ortega & A. Santos-Guerra. 2001. Phylogenetic relationships and evolution of Crassulaceae inferred from *mat*K sequence data. Amer. J. Bot. 88: 76–91.

———, ———, ———, J. Francisco-Ortega & A. Santos-Guerra. In press. Phylogenetics and diversification in the Macronesian clade (Crassulaceae) inferred from molecular and morphological data. Syst. Bot.

Olmstead, R. G., B. Bremer, K. M. Scott & J. D. Palmer. 1993. A parsimony analysis of the Asteridae sensu lato based on *rbc*L sequences. Ann. Missouri Bot. Gard. 80: 700–722.

Parkinson, C. L., K. L. Adams & J. D. Palmer. 1999. Multigene analyses identify the three earliest lineages of extant flowering plants. Curr. Biol. 9: 1485–1488.

Posada, D. & K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14: 817–818.

Qiu, Y-L., J. Lee, F. Bernasconi-Quadroni, D. E. Soltis, P. S. Soltis, M. Zanis, E. A. Zimmer, Z. Chen, V. Savolainen & M. W. Chase. 1999. The earliest angiosperms: Evidence from mitochondrial, plastid and nuclear genomes. Nature 402: 404–407.

Queiroz, A. de, M. J. Donoghue & J. Kim. 1995. Separate versus combined analysis of phylogenetic evidence. Ann. Rev. Ecol. Syst. 26: 657–681.

Queiroz, K. de & J. Gauthier. 1990. Phylogeny as a central principle in taxonomy: Phylogenetic definitions of taxon names. Syst. Zool. 39: 307–322.

——— & ———. 1992. Phylogenetic taxonomy. Ann. Rev. Ecol. Syst. 23: 449–480.

Rannala, B., J. P. Huelsenbeck, Z. Yang & R. Nielsen. 1998. Taxon sampling and the accuracy of large phylogenies. Syst. Biol. 47: 702–710.

Raven, P. H. & D. I. Axelrod. 1974. Angiosperm biogeography and past continental movements. Ann. Missouri Bot. Gard. 77: 539–673.

Rosendahl, C. O., F. K. Butters & O. Lakela. 1936. A monograph on the genus *Heuchera*. Minnesota Stud. Pl. Sci. 2: 1–180.

Sang, T., D. J. Crawford, S. G. Kim & T. F. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Amer. J. Bot. 84: 1120–1136.

Savile, D. B. O. 1953. Splash-cup dispersal mechanism in *Chrysosplenium* and *Mitella*. Science 117: 250–251.

———. 1975. Evolution and biogeography of Saxifragaceae with guidance from their rust parasites. Ann. Missouri Bot. Gard. 62: 354–361.

Savolainen, V., M. W. Chase, C. M. Morton, S. B. Hoot, D. E. Soltis, C. Bayer, M. F. Fay, A. de Bruijn, S. Sullivan & Y-L. Qiu. 2000. Phylogenetics of flowering plants based upon a combined analysis of plastid *atp*B and *rbc*L gene sequences. Syst. Biol. 49: 306–362.

Schnabel, A. & J. F. Wendel. 1998. Cladistic biogeography of *Gleditsia* (Leguminosae) based on *ndh*F and *rpl*16 chloroplast gene sequences. Amer. J. Bot. 85: 1753–1765.

Schulze-Menz, G. K. 1964. Saxifragaceae. Pp. 201–206 *in* H. Melchior (editor), A. Engler's Syllabus der Pflanzenfamilien, Gebruder Borntraeger, Berlin.

Seelanan, T., H. Schnabel & J. F. Wendel. 1997. Congruence and consensus in the cotton tribe. Syst. Bot. 22: 259–290.

Segraves, K. A. & J. N. Thompson. 1999. Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. Evolution 53: 1114–1121.

Soltis, D. E. 1980. Karyotypic relationships among species of *Boykinia, Heuchera, Mitella, Sullivantia, Tiarella*, and *Tolmiea* (Saxifragaceae). Syst. Bot. 5: 17–29.

——— & L. Hufford. In press. Clarifying the evolution of epigyny: Ovary development in Saxifragaceae. Int. J. Pl. Sci.

——— & R. K. Kuzoff. 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). Evolution 49: 727–742.

——— & P. S. Soltis. 1995. Plant molecular systematics: Inferences of phylogeny and evolutionary processes. Pp. 139–194 *in* M. K. Hecht (editor), Evolutionary Biology, Vol. 28. Plenum Press, New York.

——— & ———. 1997. Phylogenetic relationships in Saxifragaceae s. l.: A comparison of topologies based on 18S rDNA and *rbc*L sequences. Amer. J. Bot. 84: 504–522.

——— & ———. 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. Pp. 1–42 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II. Kluwer, Boston.

——— & ———. 1999. Polyploidy: Origins of species and genome evolution. Trends Ecol. Evol. 14: 349–351.

———, ———, T. G. Collier & M. L. Edgerton. 1991a. Chloroplast DNA variation within and among genera of the *Heuchera* group: Evidence for chloroplast capture and paraphyly. Amer. J. Bot. 78: 1091–1112.

———, M. S. Mayer, P. S. Soltis & M. L. Edgerton. 1991b. Chloroplast-DNA variation in *Tellima grandiflora* (Saxifragaceae). Amer. J. Bot. 78: 1379–1390.

———, D. R. Morgan, A. Grable, P. S. Soltis & R. Kuzoff. 1993. Molecular systematics of Saxifragaceae sensu stricto. Amer. J. Bot. 80: 1056–1081.

———, R. K. Kuzoff, R. Gornall & K. Ferguson. 1996a. *mat*K and *rbc*L gene sequence data indicate that *Saxifraga* (Saxifragaceae) is polyphyletic. Amer. J. Bot. 83: 371–382.

———, L. A. Johnson & C. Looney. 1996b. Discordance between ITS and chloroplast topologies in the *Boykinia* group (Saxifragaceae). Syst. Bot. 21: 169–185.

———, P. S. Soltis, M. E. Mort, M. W. Chase, V. Savolainen, S. B. Hoot & C. M. Morton. 1998. Inferring complex phylogenies using parsimony: An empirical approach using three large DNA data sets for angiosperms. Syst. Biol. 47: 32–42.

———, P. S. Soltis, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanis, V. Savolainen, W. H. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, L. M. Prance, W. John Kress, K. C. Nixon & James S. Farris. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbc*L, and *atp*B sequences. Bot. J. Linn. Soc. 133: 381–461.

———, M. Tago-Nakazawa, Q.-Y. Xiang, S. Kawano, J. Murat & M. Wakabayashi. 2001. Phylogenetic relationships and evolution in *Chrysosplenium* (Saxifragaceae) based on *mat*K sequence data. Amer. J. Bot. 88: 883–893.

Soltis, P. S., D. E. Soltis & M. W. Chase. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402: 402–404.

Spongberg, S. A. 1972. The genera of Saxifragaceae in the Southeastern United States. J. Arnold Arbor. 53: 409–494.

Stuessy, T. F. 2000. Taxon names are not defined. Taxon 49: 231–233.

Sullivan, J. 1996. Combining data with different distributions of among-site rate variation. Syst. Biol. 45: 375–380.

Swofford, D. L. 1999. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b4a. Sinauer, Sunderland, Massachusetts.

Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl. Molec. Biol. 17: 1105–1109.

Thompson, J. D., D. G. Higgins & T. J. Gibson. 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22: 4673–4680.

———, T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins. 1997. The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24: 4876–4882.

Thompson, J. N. 1994. The Coevolutionary Process. Univ. Chicago Press, Chicago.

——— & O. Pellmyr. 1992. Mutualism with pollinating seed parasites amid co-pollinators: Constraints on specialization. Ecology 73: 1780–1791.

Webb, D. A. & R. J. Gornall. 1989. A Manual of Saxifragas and their Cultivation. Timber Press, Portland.

Wen, J. & E. A. Zimmer. 1996. Phylogeny of *Panax* L. (the ginseng genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. Molec. Phylogenet. Evol. 6: 167–179.

Wendel, J. F. & J. J. Doyle. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. Pp. 265–296 *in* D. E. Soltis, P. S. Soltis & J. J. Doyle (editors), Molecular Systematics of Plants II. Kluwer, Boston.

Wheeler, W. C., J. C. Gatesby & R. Desalle. 1995. Elision: A method for accommodating multiple molecular sequence alignments with alignment-ambiguous sites. Molec. Phylogenet. Evol. 4: 1–9.

Wu, C.-Y. & T.-C. Ku. 1992. A new tribe with a new monotypic genus of Saxifragaceae (s.l.) from China. Acta Phytotax. Sin. 30: 193–196.

Yang, Z. 1994. Phylogenetic analysis using parsimony and likelihood methods. J. Molec. Evol. 39: 294–307.

Appendix.

Definitions of Taxon Names

We consulted the phylogenetic system of nomenclature proposed by de Queiroz and Gauthier (1992) and the rules provided in the PhyloCode (http://www.ohiou.edu/phylocode/). The definitions provided below are all node based. The reference phylogenies for the *Heuchera* and *Boykinia* groups are provided in Soltis and Kuzoff (1995), Kuzoff (1998), and Soltis et al. (1996b), respectively. Reference phylogenies for *Saxifraga* and *Micranthes* are provided in Soltis et al. (1996b) and Mort and Soltis (1999). Letters given to clades correspond to those given in Figure 4 and in Table 5. The names presented (see also Fig. 4, Table 5) are not correspondent to nomenclatural equivalents under the ICBN (Greuter et al., 2000), but are applied following the Phylocode.

Saxifragaceae (clade A). The least inclusive clade containing *Saxifraga granulata* L., *Saxifraga mertensiana, Saxifragella bicuspidata, Saxifraga tolmiei, Heuchera americana* L., *Darmera peltata, Peltoboykinia tellimoides, Cascadia nuttallii, Tanakaea radicans, Astilbe rivularis* Buch.-Ham. ex D. Don, and *Jepsonia parryi.*

Saxifraga (clade B). The least inclusive clade containing *Saxifraga granulata, Saxifraga mertensiana, Saxifragella bicuspidata, Saxifraga tricuspidata,* and *Saxifraga cernua.*[a]

Micranthina (clade C). The least inclusive clade containing *Micranthes semipubescens* Sweet, *Saxifraga tolmiei, Heuchera americana, Darmera peltata, Peltoboykinia tellimoides, Cascadia nuttallii, Tanakaea radicans, Astilbe rivularis,* and *Jepsonia parryi.*

Cascadina (clade D). The least inclusive clade containing *Cascadia nutallii* and *Saxifragodes albowiana.*

Micranthes (clade E). The least inclusive clade containing *M. semipubescens, M. tolmiei, M. ferruginea* Graham, *M. howellii* (Greene) Small, and *M. odontoloma* (Piper) A. Heller.[b]

Peltoboykinina (clade F). The least inclusive clade that contains *Peltoboykinia tellimoides* and *Chrysosplenium oppositifolium* L.

Darmerina (clade G). The least inclusive clade that contains *Darmera peltata, Mukdenia rosii,* and *Bergenia cordifolia.*

Heucherina (clade H). The least inclusive clade that contains *Tiarella cordifolia* L., *Elmera racemosa* (S. Watson) Rydb., *Heuchera americana* L., *Lithophragma parviflorum* (Hook.) Nutt. ex Torr. & A. Gray, *Tolmiea menziesii* (Pursh) Torr. & A. Gray, and *Mitella diphylla* L.[c]

Boykinina (clade I). The least inclusive clade that contains *Jepsonia parryi, Boykinia aconitifolia* Nutt., and *Sullivantia sullivantii* (Torr. & A. Gray) Britton.

Astilbina (clade J). The least inclusive clade that contains *Astilbe rivularis* and *Saxifragopsis fragariodes.*

Leptarrhenina (clade K). The least inclusive clade that contains *Leptarrhena pyrolifolia* and *Tanakaea radicans.*[d]

[a] This *Saxifraga* clade corresponds to *Saxifraga* sects. *Ciliatae, Cymbalaria, Irregulares, Porphyrion, Ligulatae,*

Xanthizoon, *Trachyphyllum*, *Gymnopera*, *Cotylea*, *Odontophyllae*, *Mesogyne*, and *Saxifraga*, but not sections *Merkianae* and *Micranthes* (sects. after Gornall, 1987; Webb & Gornall, 1989).

[b]The *Micranthes* clade is equivalent to *Saxifraga* sects. *Micranthes* and *Merkianae* of Gornall (1987). The name *Micranthes* has been used in the past to refer to species of both sections *Micranthes* and *Merkianae*. Gornall (1987; see also Webb & Gornall, 1989) segregated section *Merkianae* from section *Micranthes*. Haworth (1812) established the segregate genus *Micranthes*.

[c]*Mitella* is clearly polyphyletic; although all species are within the *Heuchera* group clade, it is still unclear how they are related and how best to treat them taxonomically.

[d]This clade corresponds to the Leptarrheneae of Schulze-Menz (1964).

REVISION OF *RHYNCHOSTEGIOPSIS* (LEUCOMIACEAE: MUSCI)[1]

Michelle Price[2]

Abstract

Rhynchostegiopsis Müll. Hal. is a morphologically variable genus of five species that grow in moist, humid habitats in montane forests in Mexico, Central America, the Caribbean, tropical Andes, and southeastern Brazil. This moss genus is characterized by ecostate leaves with serrate margins and lax elongate median cells; two-celled axillary hairs; furrowed exostome teeth that are horizontally striate below; endostomial cilia; rostrate opercula; and cucullate calyptrae. The genus lacks differentiated alar cells, paraphyllia, pseudoparaphyllia, a central strand, and hyalodermis. Several of its key morphological features are found in different families: the Hypnaceae, Hookeriaceae, and Leucomiaceae. In this study, *Rhynchostegiopsis* is retained in the Leucomiaceae based on overall sporophyte and gametophyte morphology, including its stem anatomy, as well as the presence of cucullate calyptrae.

Key words: Hookeriales, Leucomiaceae, morphology, *Rhynchostegiopsis*, taxonomy.

Rhynchostegiopsis is a small pleurocarpous moss genus of five species (*R. brasiliensis* Broth., *R. carolae* Crosby, *R. costaricensis* H. Rob. & D. G. Griffin, *R. flexuosa* (Sull.) Müll. Hal., and *R. tunguraguana* (Mitt.) Broth.) that are restricted to the Neotropics. *Rhynchostegiopsis*, at first glance, appears to be a lax-celled hypnoid genus. It lacks: costae; differentiated alar cells; cell ornamentation; paraphyllia; pseudoparaphyllia; a central strand; and hyalodermis. These "absent" character states help define *Rhynchostegiopsis* in combination with the presence of long, lax leaf cells; serrate leaf margins; two-celled axillary hairs (Fig. 1d); stems composed of large thin-walled cells throughout (Fig. 1c); collenchymatous exothecial cells; furrowed, horizontally striate exostome teeth (Fig. 1a); endostomial cilia (Fig. 1b); and cucullate calyptrae (Fig. 1e).

Decisions on the ordinal placement of *Rhynchostegiopsis* have been problematic. This is because *Rhynchostegiopsis* shares many of its key morphological features with three families from two large orders of mosses, the Hookeriaceae s.l. and Leucomiaceae in the Hookeriales, and the Hypnaceae in the Hypnales. The cucullate calyptrae and endostomial cilia of *Rhynchostegiopsis* are morphological features that are typical of genera in the Hypnaceae. Characters of Hookeriales that are shared by *Rhynchostegiopsis* are the furrowed exostome teeth; two-celled axillary hairs; undifferentiated alar cells; and a lack of pseudoparaphyllia, a central strand, and hyalodermis. Based on sporophyte and gametophyte morphology, including stem anatomy and the presence of cucullate calyptrae, *Rhynchostegiopsis* is currently recognized as belonging to the Leucomiaceae in the Hookeriales.

Generic Placement of *Rhynchostegiopsis*

The Hookeriales are a large and morphologically variable order of mosses recognized by trends in characters rather than exclusive character traits that can be used to define its members. The current classification and systematic understanding of the Hookeriales remains rather unsatisfactory, mainly due to the variability of character states and high degree of homoplasy within the order. Hedenäs (1996a) presented a tabular overview of the major classification systems of the Hookeriales since Brotherus (1925). These classification schemes, from Brotherus (1925) to Whittemore and Allen (1989), differ in the circumscription and recognition of families in the order (ranging from 2 to 8 families), the positioning of genera, and in the characters considered phylogenetically informative (either mostly sporophyte or both sporophyte and gametophyte characters). Buck (1991) discussed the use of sporophytic versus gametophytic characters in the classification schemes of pleurocarpous

[1] Support for this study is gratefully acknowledged from the National Science Foundation, grant 9522034 (PEET: Partnerships for Enhancing Expertise in Taxonomy). I thank the curators of BM, DUKE, E, H, H-BR, LPB, MO, NY, and US for loan of the specimens used in this study, and all the individuals who helped in my efforts to complete this work, particularly Bruce Allen, Steve Churchill, Victoria Hollowell, Robert Magill, and three anonymous reviewers for comments on earlier drafts of this manuscript. I also thank Christina Casado for assistance with the GIS work, and my advisors, Robert Magill and Steve Churchill, for their help and encouragement.

[2] Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A. michelle.price@mobot.org

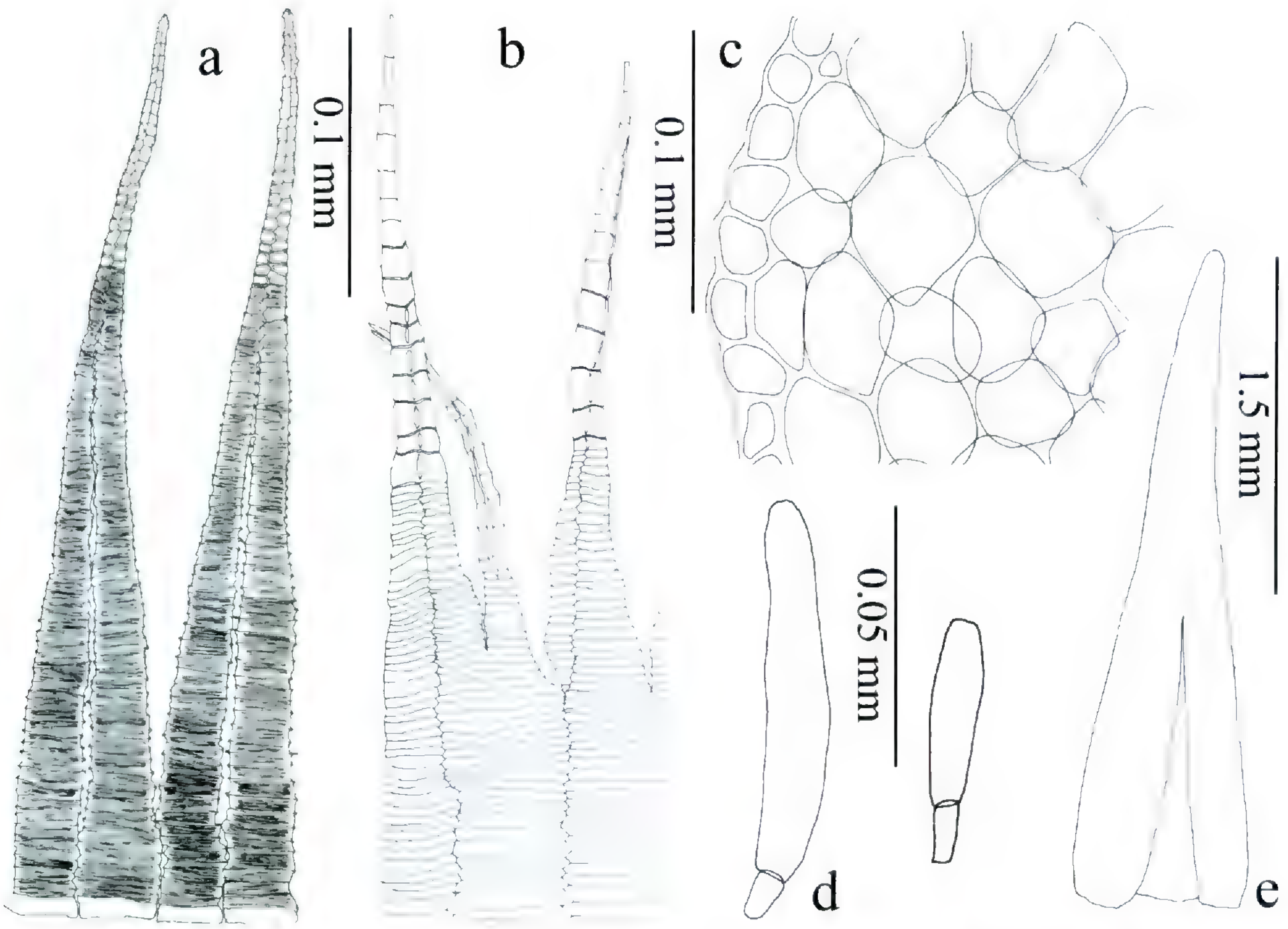


Figure 1. Diagnostic features of the genus *Rhynchostegiopsis*. Outer surface of (a) exostome, and (b) endostome of *R. flexuosa*. —c. Stem cross section of *R. tunguraguana*. —d. Axillary hairs from *R. tunguraguana* and *R. brasiliensis*, respectively. —e. Calyptra of *R. tunguraguana*. (a, b: *Morton 7395*, US; c: *Churchill & Sastre de Jesús 12999*, MO; d: *Schiffner 1454*, BM, isolectotype, and *Lewis 83–710*, LPB; e: *Churchill & Sastre de Jesús 12999*, MO.)

mosses and the implications this has on the interpretation of systematic relationships within this large group. In the mosses the gametophyte is the dominant phase of the life cycle. It was concluded that focus placed on the sporophyte alone could be misleading, and that phylogenetic relationships are more likely to be revealed by overall gametophytic correlations. Differential weight given to either gametophytic or sporophytic characters in *Rhynchostegiopsis*, in accordance with any particular classification scheme, has been important in influencing the systematic placement of the genus over time.

Utilizing mostly sporophytic features, Fleischer (1906–1908) divided the Hookeriaceae into four tribes. He placed *Rhynchostegiopsis* in his Hypnelleae between *Sauloma* (Hook. f. & Wilson) Mitt. and *Philophyllum* Müll. Hal., based on similarities in peristome morphology. Brotherus (1925) adopted Fleischer's general systematic scheme. He maintained the same concept of the Hypnelleae but raised it to the level of subfamily. The Fleischer-Brotherus system was primarily based on Philibert's observations of peristome morphology (see Taylor, 1962, for reference to entire series; Crosby, 1974; Buck, 1991). In contrast to the previous sporophyte-based classifications, Miller (1971) published an overview of the Hookeriales based mainly on gametophyte morphology and using a set of 23 classification principles influenced by the work of Hutchinson (1959). This phylogenetic arrangement of the genera in the Hookeriales differed from all previous work. Miller (1971) was the first to recognize the similarity between *Rhynchostegiopsis* and *Leucomium* Mitt. The latter genus differed from *Rhynchostegiopsis* only by its entire leaf margins, lack of endostomial cilia, and sexuality. *Leucomium* is autoicous, synoicous, or polyoicous (Allen, 1987), whereas *Rhynchostegiopsis* is dioicous. *Rhynchostegiopsis* was placed in the Leucomiaceae by Miller (1971), along with *Philophyllum*, *Sauloma*, *Pulvinella* Broth. & Herzog, *Stenodesmus* (Mitt.) A. Jaeger (Miller, 1971), and the two original members of the family, *Leucomium* and *Vesiculariopsis* Broth. However, Miller gave no explanation of the concept of this newly expanded family in this paper. Allen (1987), in a revision of *Leucomium*, documented the history related to the development of the concept of this genus, the family Leucomiaceae, and the different interpretations of the systematic relationships therein. The close *Leucomium–Rhynchostegiopsis* relationship was supported in this work based on the overall morphological

similarity of these two genera, and they were considered to be the only members of the Leucomiaceae at that time (Allen, 1987).

Crosby (1974) published a new classification of the Hookeriaceae based on his Philibert-Dixon principle, using peristome form and subsequently gametophytic characters to group genera (Dixon, 1932; Crosby, 1974). The Philibert-Dixon principle was defined as primary weight being given to characters of the sporophyte, in particular the peristome, when constructing a classification for the mosses (Crosby, 1974). Based on this principle, mosses with similar gametophytes must not be grouped together if their sporophytes differ; instead they should be placed with mosses that have similar peristomes. The Hookeriaceae were divided into eight subgroups: the Distichophylloid, Adelothecioid, Cyathophoroid, Hookerioid, Hemiragoid, Hookeriopsoid, Pilotrichidioid, and Ephemeropsid groups (Crosby, 1974). *Rhynchostegiopsis* was placed in Crosby's Hemiragoid group along with *Hemiragis* (Brid.) Besch., *Philophyllum*, *Dimorphocladon* Dixon, and *Sauloma*. This Hemiragoid group was defined by lanceolate leaves, none or two costae, and lax laminal cells. Crosby (1974) found that within the Hookeriaceae, two characters of the peristome were variable: (1) the median line of the exostome (either furrowed or zig-zag), and (2) endostomial cilia (either present or absent). Mosses with, or mosses without, endostomial cilia did not form natural groups. It was considered that any arrangement of taxa based on either of those character states would result in groups with low overall morphological similarity (Crosby, 1974). One of the few features that differs between *Rhynchostegiopsis* and *Leucomium* is the presence of endostomial cilia found in *Rhynchostegiopsis*. In contrast to previous classifications, *Leucomium* was placed in the Hypnaceae near *Vesicularia* (Müll. Hal.) Müll. Hal. based on its cucullate calyptra and leaf areolation. However, the family Leucomiaceae was synonymized with the Hookeriaceae (Crosby, 1974). This placement of *Leucomium* in the Hypnaceae was later followed by Vitt (1984) in his classification of the Bryopsida.

Comparison of members of the Leucomiaceae to some genera of the Hypnaceae indicates that there are less than clear distinctions between these families. Both families exhibit an overlap in characters and character state variation in sporophyte and gametophyte morphology. This broad overlap in characters between the Leucomiaceae and Hypnaceae is exemplified by the genera *Rhynchostegiopsis*, *Leucomium*, and *Vesicularia*. Both *Leucomium* and *Rhynchostegiopsis* (Leucomiaceae) resemble *Vesicularia* (Hypnaceae) in habit and the presence of a cucullate calyptra, but they both lack stem pseudoparaphyllia, which are typical of the Hypnaceae. All three genera are ecostate, and leaf areolation in *Leucomium* and *Rhynchostegiopsis* is similar to that of *Vesicularia*. *Vesicularia* and *Rhynchostegiopsis* both have endostomial cilia, while *Leucomium* lacks them. Discordant with the Hypnales but typical of members of Hookeriaceae, sensu Whittemore and Allen (1989), are the lack of pseudoparaphyllia and the two-celled axillary hairs of *Rhynchostegiopsis* and *Leucomium*.

In contrast to previous sporophyte-based classification systems, and that of Miller (1971), Buck and Vitt (1986) presented a familial classification of the pleurocarpous mosses based on correlated gametophytic and sporophytic characters. The Hookeriales were defined by leaves with lax areolation; costa single, but more typically absent or double; collenchymatous exothecial cells; an exostome with a median furrow; rostrate opercula; and mitrate calyptrae. The Leucomiaceae were distinguished from other families in the order by their elongate, lax leaf cells, enlarged cortical cells of the stem, and cucullate calyptrae. The family contained just three genera: *Leucomium* and *Vesiculariopsis*, following the arrangement of Brotherus (1925), and *Rhynchostegiopsis*. Buck (1987, 1988), in further rearrangements of taxa within the Hookeriales, divided the order into five families according to gametophytic characters. He considered the variation in exostome form in the Hookeriales to be an unreliable indicator of relationships both within and between families. *Rhynchostegiopsis* was retained with *Leucomium*, in his Leucomiaceae, based on gametophytic similarities. The Leucomiaceae were characterized by ecostate leaves with long-lax leaf cells; fragile stems; undifferentiated stem anatomy; a typical hookerioid peristome (except for endostomial cilia found in some taxa); and cucullate calyptrae (Buck, 1987). The characters of cucullate calyptra and undifferentiated stem anatomy of the Leucomiaceae were used to segregate it from the Hookeriaceae.

Whittemore and Allen (1989) presented a classification of the Hookeriales based on gametophyte and sporophyte morphology, recognizing only two groups within the Hookeriales, the *Hookeria* group (as the Hookeriaceae Schimp.) and the *Daltonia* group (as the Daltoniacaeae Schimp.). The Leucomiaceae were placed in synonymy with their expanded Hookeriaceae. This broader concept of Hookeriaceae was defined as a taxon with branched stems; bicostate or ecostate leaves; straight rhizoids (unbranched and tightly clustered below the leaf

base); two-celled axillary hairs; and a weakly pigmented stem cortex (Whittemore & Allen, 1989).

Despite the uncertainty over the recognition of the Leucomiaceae expressed by some of the earlier-mentioned authors, the concept of this family has been developed over time. Buck (1992) placed the neotropical endemic genus *Philophyllum* in the Leucomiaceae supported by its distinctive stem anatomy, calyptra, and annular development. *Philophyllum* has calyptrae that are variable in state; they are both mitrate and cucullate. Although members of the Leucomiaceae are considered defined, in part, by cucullate calyptrae, the infraspecific variability in calyptra form in *Philophyllum* was not considered grounds for its exclusion. *Philophyllum* is similar to all other members of the Leucomiaceae in its characteristic large thin-walled stem cells. Buck (1992) noted that the stem anatomy in the Leucomiaceae was unique to this family and an important defining character of it. Although leaves in the Leucomiaceae are generally ecostate, the occasional occurrence of short, double costae in *Philophyllum* was not considered critical to exclude the genus from the Leucomiaceae (Buck, 1992). Short double costae have been reported in some specimens of *Rhynchostegiopsis tunguraguana* Buck (1998). However, in this study only traces of a bistratose leaf lamina have been seen in a few specimens indicating that the occurrence of double costae is in fact rare. These traces of a bistratose layer were seen at the very base of leaves and were only visible in cross section.

The Ptychomniaceae have cucullate calyptrae in common with the Leucomiaceae. The Ptychomniaceae were first placed in the Hookeriales, near the Hookeriaceae, by Robinson (1971). It was considered close to the Hookeriaceae and Leucomiaceae based on the furrowed exostome teeth of some of the genera, the lack of costae, and poorly developed alar cells (Robinson, 1975). Hattaway (1984), in a monograph of the Ptychomniaceae, also concluded that this family belonged in the "Hypno-bryalean" order close to the Hookeriaceae, Sematophyllaceae, or Hypnaceae, although the exact affinities of it were not fully stated. Characters of the Ptychomniaceae that differ from those of the Leucomiaceae are stem paraphyllia, colored alar cells that can be differentiated, incrassate-porose cells throughout, and ribbed capsules (Hattaway, 1984). All these features are, in fact, generally discordant with most genera in the Hookeriales, and more specifically all those in the Leucomiaceae. If the Ptychomniaceae are excluded from the Hookeriales, pending further investigation, then the Leucomiaceae are the only family in the Hookeriales with cucullate calyptrae.

The genus *Tetrastichium*, containing *Tetrastichium fontanum* (Mitt.) Cardot, was traditionally considered to be a member of the Hookeriaceae based on its short, hexagonal, lax upper leaf cells (Allen et al., 1985b). After closer examination of this species, Allen et al. (1985b) discussed the systematic placement of it. They were the first to note the similarity between *T. fontanum* and *Rhynchostegiopsis*, especially in leaf cell areolation and peristome features. The exostome and endostome of these two genera are almost identical. Another species, *Lepidopilum virens* Cardot, was later transferred to *Tetrastichium* by Churchill (1989) based on its leaf cell areolation, as well as its endostome and exostome features. Morphological characters traditionally used to align *Tetrastichium fontanum* with *Hookeria* Sm. in the Hookeriaceae included leaf cell areolation and lack of a costa. Ecostate leaves are also characteristic of the Leucomiaceae. In addition to this, *Tetrastichium fontanum* has leaf cells similar to those of *Rhynchostegiopsis brasiliensis*, the larger forms of *R. flexuosa*, and *Leucomium*. *Tetrastichium* is another genus that is variable in its calyptra form, which can be either mitrate or cucullate, a feature that it shares with *Philophyllum*. Inclusion of the genus *Tetrastichium* in the Leucomiaceae is therefore supported by leaf areolation, stem anatomy, exostome and endostome features, and its calyptra.

Hedenäs (1995) investigated relationships among the diplolepidous pleurocarpous mosses, and between the Hookeriales and Sematophyllaceae (Hedenäs, 1996b) using cladistic methods. *Rhynchostegiopsis* was not included in the data sets; however, *Leucomium* was found to be a relatively basal member of the "Hookeriales" along with *Tetrastichium virens* (Cardot) S. P. Churchill and *Tetrastichium fontanum*. In an additional paper Hedenäs (1996a) presented a set of cladistic analyses of "Hookeriales" based on 75 morphological characters of 80 species from 50 genera, this time including 1 species of *Rhynchostegiopsis*. The species *Leucomium strumosum* (Hornsch.) Mitt. and *Rhynchostegiopsis flexuosa*, and *Tetrastichium fontanum* and *T. virens* consistently formed sister clades in the analyses, supporting the proposed relationships of *Leucomium–Rhynchostegiopsis* and *Tetrastichium–Leucomium–Rhynchostegiopsis*. However, the topologies of the trees from both these sets of analyses were not well supported statistically (Hedenäs, 1995, 1996b). The separate analyses also highlighted some of the problems associated with the high levels of homoplasy within the Hookeriales and Hypnales, especially how these significant levels of homoplasy may influence results in large-

scale cladistic analyses when using large numbers of taxa and/or characters.

To date, taxonomic and floristic treatments differ in the recognition of the Leucomiaceae and positioning of *Rhynchostegiopsis* within the Hookeriales. Authors either follow the more traditional view of *Rhynchostegiopsis* aligned with the Hookeriaceae (Bartram, 1949; Crum & Steere, 1957; Crum & Bartram, 1958; Whittemore & Allen, 1989), or recognize the Leucomiaceae and the *Rhynchostegiopsis–Leucomium* relationship within it (Allen, 1987; Crum, 1994; Churchill & Linares, 1995; Buck, 1998). The Leucomiaceae are defined herein by ecostate or sometimes bicostate leaves; lax leaf cells; stems composed of large thin-walled cells throughout; collenchymatous exothecial cells; rostrate opercula; striate-furrowed exostome teeth; a well-developed endostome, with or without cilia; and cucullate or mitrate calyptrae. It is clear that *Rhynchostegiopsis* belongs in the Leucomiaceae, along with *Leucomium*, *Philophyllum*, and *Tetrastichium* (Mitt.) Cardot, based on the overall sporophytic and gametophytic characters they share and that define the Leucomiaceae. Other genera that were traditionally placed in the Leucomiaceae have been transferred to other families, mainly the Pilotrichaceae (*Vesiculariopsis*, *Sauloma*, and *Stenodesmus*).

Morphological Variability in *Rhynchostegiopsis*

Study of over 400 herbarium specimens allowed for morphological variability in the genus to be assessed throughout its geographic range. Species were identified using measurements of leaf cell length, leaf size, and stem size combined with leaf shape, apex length and shape, and upper leaf margin form. *Rhynchostegiopsis* is a difficult genus because there are few characters that define it, and its characters can vary continuously within and between species. Variation within species is not obvious when comparing only type specimens or the typical forms of each taxon. However, often striking differences in plant form, size, and leaf morphology emerge when examining a large number of specimens. As a result of the variability in characters as well as the wide range of variation seen within species of *Rhynchostegiopsis*, the circumscription of them is difficult. Critical examination of leaves from different positions on each plant may be required for identification purposes.

Character variations in *Rhynchostegiopsis* often appear to be independent of the other characters, and their states, for a particular species. For any given species, specimens that may be unusual in one character have other characters typical for that species. *Rhynchostegiopsis* grows in moist and humid habitats. Morphological differences can be seen when plants are found growing on different substrates and under presumably different environmental regimes. Such variability has also been attributed to environmental influences in other genera of the Hookeriales, *Leucomium* (Allen, 1987), *Pilotrichidium* Besch. (Allen & Crosby, 1986), *Stenodictyon* (Allen et al., 1985a), and *Philophyllum* (Buck, 1992).

Phytogeography

Rhynchostegiopsis is endemic to the Neotropics. One species, *Rhynchostegiopsis brasiliensis*, is especially limited in distribution, as it is known only from three localities in southeastern Brazil (represented by the triangle in Fig. 2). The other species are found in Mexico, Central America, the Caribbean, and the northern and central Andes. This tropical Andes–southeast Brazil disjunct distribution pattern is found frequently in mosses (Buck, 1990), including the closely related genus *Leucomium* (Allen, 1987). *Rhynchostegiopsis tunguraguana* is the most geographically widespread of the species and is found from Mexico through Central America to northern South America and Bolivia (Fig. 2). No collections of *R. tunguraguana* have been seen from Peru, but this is probably due to a lack of collecting rather than its absence from Peru. In the Caribbean, *R. tunguraguana* is known only from Jamaica and the Dominican Republic.

The most frequently collected species, *R. flexuosa*, is almost entirely restricted to Central America and the Caribbean. In South America *R. flexuosa* is known from two collections in Colombia (*Steere 7688a* and *7671*) (Fig. 3a). Widespread in the Caribbean, *R. flexuosa* is found on almost all the islands from Cuba to Trinidad. The remaining species, *R. costaricensis* and *R. carolae*, are narrow Central American endemics (Fig. 3b and c, respectively). They are both known from Costa Rica with one locality in Honduras for *Rhynchostegiopsis costaricensis*, and one locality in Panama for *Rhynchostegiopsis carolae*.

Materials and Methods

This revision is based on 406 specimens from BM, DUKE, F, H, H-BR, LPB, MO, NY, and US. All specimens examined were entered into the TROPICOS–MOST database at Missouri Botanical Garden (http://www.mobot.org). Locality information and coordinates were included with each spec-

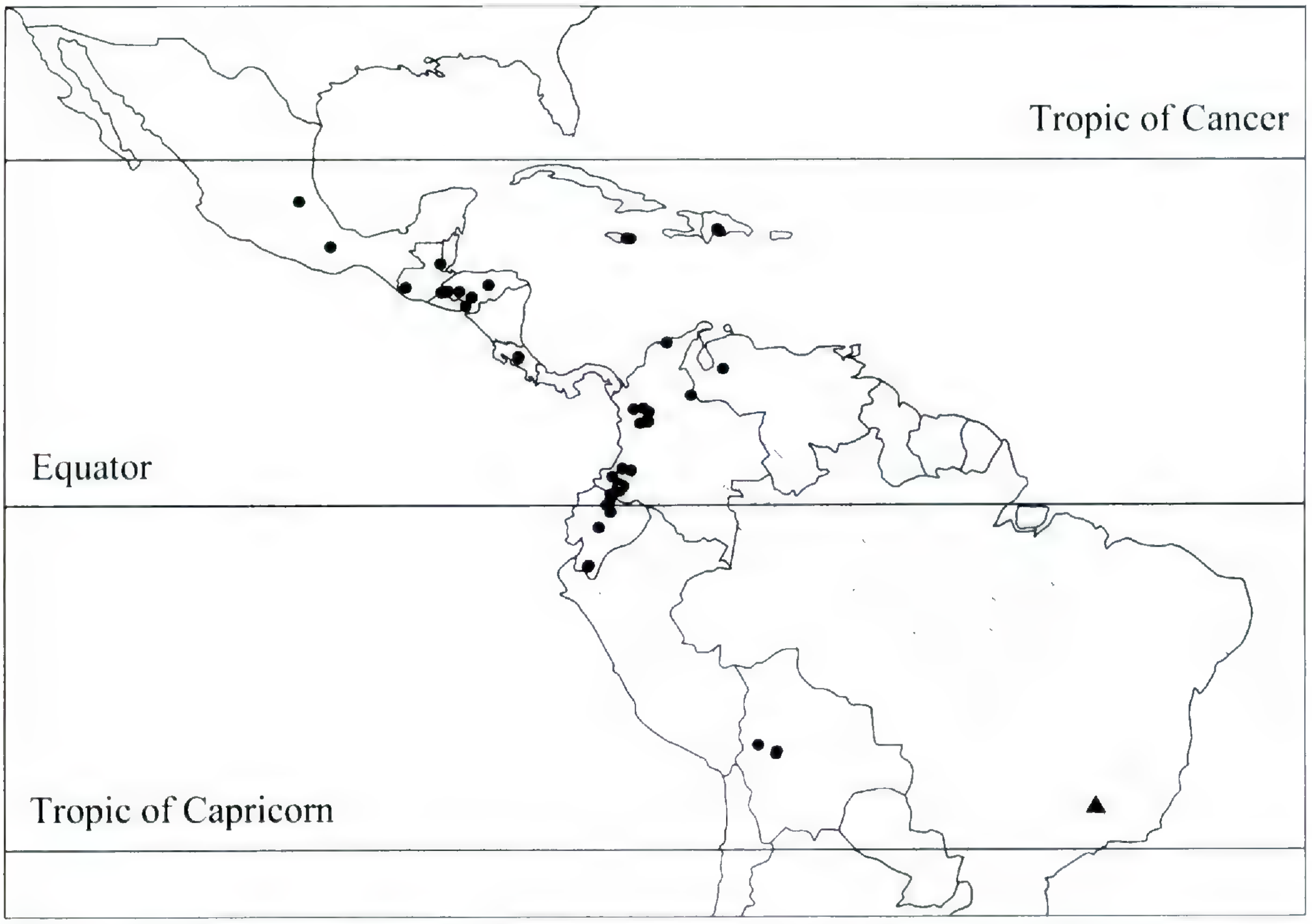


Figure 2. Distribution of *Rhynchostegiopsis brasiliensis* (triangle) and *Rhynchostegiopsis tunguraguana* (circles).

imen entry. Mapping was done using ArcView GIS (ESRI, 1998). Coordinates for localities were extracted from the TROPICOS–MOST database, and distribution maps are based on the herbarium specimens examined during this study. The sections on "selected specimens examined" include one collection per political subdivision (province) for each country. The complete list of examined specimens is available in Appendix 1 or through Internet access to the TROPICOS–MOST database. Specimens were examined in aqueous and Hoyer's solution mounts (Anderson, 1954), with cell measurements taken from Hoyer's solution mounts. Leaf width is measured at mid-leaf. Measurements of the setae include the vaginula. Cell measurements include the cell wall. Marginal cells refer to the outermost row of cells of the leaf lamina. Median cells refer to the cells that are central in the leaf.

Taxonomic Treatment

Rhynchostegiopsis Müll. Hal., Nuovo Giorn. Bot. Ital. n.s. 4: 163: 1897, as "tribus." TYPE: *Rhynchostegiopsis complanata* Müll. Hal.

Plants small to large, 10–120 mm long, glossy, pale or dark green to yellow-green or golden with age; forming dense to thin mats. *Stems* fragile to sturdy, sometimes dark colored, creeping or spreading, sub-pinnately to irregularly branched, complanate-foliate; in cross section cells homogeneous, large and thin-walled, central strand absent, hyalodermis lacking; paraphyllia and pseudoparaphyllia absent; axillary hairs two-celled, basal cell short-cylindrical, small, brown, upper cell long-cylindrical, enlarged, hyaline; *rhizoids* dark red, smooth, clustered on underside of stem, at base of and below leaf insertions. *Leaves* spreading, sometimes falcate, usually crowded, not much contorted wet or dry, sometimes longitudinally folded; lanceolate, oblong-lanceolate, ovate or ovate-lanceolate, plane to slightly concave below, plane above, apices short- to long-acuminate, straight or flexuose; symmetric, not decurrent; lateral, dorsal, and ventral leaves somewhat differentiated, lateral and dorsal leaves smaller; lamina unistratose; margins entire below, sharply serrate, serrate, or serrulate above, sometimes sub-entire in some leaves; ecostate; laminal cells smooth, walls non-porose; median leaf cells lax to firm, long-linear to long-hexagonal throughout; alar cells not differentiated; basal cells slightly broader and shorter than median cells, sometimes differentiated in association with leaf gemmae; upper marginal cells conspicuously or slightly enlarged in comparison to median cells. *Asexual reproduction* none or by leaf gemmae, gemmae in distinct clusters on dorsal leaf surface, or

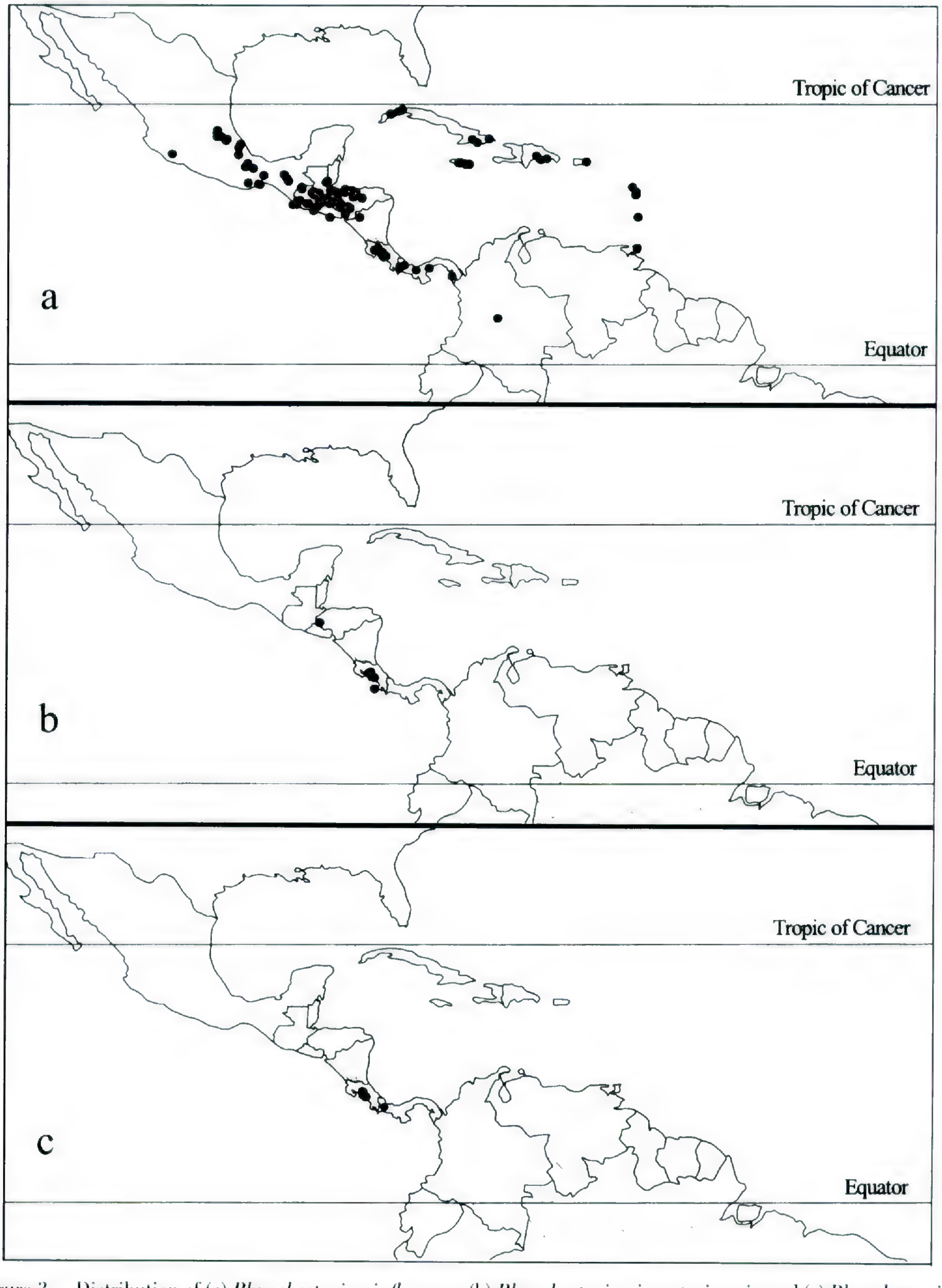


Figure 3. Distribution of (a) *Rhynchostegiopsis flexuosa*, (b) *Rhynchostegiopsis costaricensis*, and (c) *Rhynchostegiopsis carolae*.

on the stem at leaf base. *Dioicous*. *Perigonia* lateral, leaves smaller than but otherwise similar to perichaetial leaves. *Perichaetia* lateral, conspicuous or inconspicuous; leaves erect with spreading apices, ovate to ovate-lanceolate, apices sub-entire to serrate, sometimes flexuose; ecostate; median cells long-hexagonal to long-linear; basal cells shorter and broader than median cells; alar cells not differentiated. *Setae* solitary, long, smooth to sometimes roughened just below base of capsule, reddish to dark red. *Capsules* exserted, inclined to horizontal or pendent; *urn* cylindrical, smooth, neck undifferentiated; exothecial cells sub-quadrate to short-rectangular, collenchymatous with horizontal

walls usually thicker than vertical walls; stomata at base of urn, superficial; *opercula* long-rostrate from a conic base; *annuli* 2–5 layers of thin-walled cells, deciduous. *Peristome* double, exostome teeth 16, narrowly triangular, finely papillose above, densely horizontally striate with narrow median line below; endostome basal membrane high, segments 16, narrowly triangular, almost as long as exostome teeth, keeled, smooth to finely papillose, often narrowly perforate, cilia 1 to 2(3), well developed. *Calyptrae* long cucullate, often split from the base 2/3 of length, smooth. *Spores* spherical, 11–20 μm diam., finely papillose.

Etymology. *Rhynchostegiopsis* was derived from the generic name *Rhynchostegium* Bruch & Schimp., "*opsis*" meaning resembling, in reference to the similarity of its rostrate opercula to that of the genus *Rhynchostegium*. The name *Rhynchostegium* was derived from the Greek "*rhychos*," meaning beaked, and "*stege*," meaning cap or cover, in reference to the shape of its opercula.

Distribution. Central America (all countries from Mexico to Panama), the Caribbean (Cuba, Jamaica, Puerto Rico, Dominican Republic, Leeward and Windward Isles), and South America (Venezuela, Colombia, Ecuador, Bolivia, and southeastern Brazil) (Figs. 2, 3).

Ecology. Inhabiting montane forests from middle to high elevations. On trunks, roots, and branches of living trees, on fallen decayed logs and soil or leaf litter in moist, humid habitats.

Discussion. The name *Rhynchostegiopsis* was first invalidly published, as a section, in a description of a new species, *Vesicularia auricolor* Müll. Hal. (as *Vesicularia* (*Rhynchostegiopsis*) *auricolor*) (Müller, 1897a). *Rhynchostegiopsis* was established, in a slightly later publication, for the newly described *R. complanata* Müll. Hal. from Bolivia (Müller, 1897b). Two species, *Hypnum flexuosum* Sull. and *Vesicularia auricolor*, were transferred from their respective genera into the new genus *Rhynchostegiopsis* in the later publication (Müller, 1897b). A formal generic description of *Rhynchostegiopsis* was lacking in the original publication (Müller, 1897b), and the description of *R. complanata* cannot be used in its place because the genus was not monotypic when published. Instead, a small diagnosis of a "tribus" in the original (1897b) publication has been traditionally accepted as the generic description of *Rhynchostegiopsis* (Welch, 1966). This diagnosis by Müller (1897b) succinctly describes *Rhynchostegiopsis* as having serrate, ecostate leaves with lax pellucid leaf cells, a bent, fine leaf apex, and a rostrate operculum.

KEY TO THE SPECIES OF *RHYNCHOSTEGIOPSIS*

1. Plants up to 30(–40) mm long; leaves 1.0–2.5 mm long, 0.2–0.9 mm wide 2
1′. Plants up to 100(–120) mm long; leaves 1.7–7.0 mm long, 0.7–2.1 mm wide 4
2(1). Leaf apices short-acuminate ..... 1. *R. brasiliensis*
2′. Leaf apices long-acuminate 3
3(2). Leaf apices flexuose, sharply serrate, serrate or serrulate, marginal cells larger than median cells, forming a distinct border; gemmae none ..... 4. *R. flexuosa*
3′. Leaf apices not flexuose, serrate, serrulate to sub-entire, marginal cells undifferentiated, not forming a distinct border; gemmae frequent, on dorsal surface of leaf ..... 3. *R. costaricensis*
4(1). Leaves 1.7–4.6 mm long, 0.7–1.6 mm wide, margins serrate or serrulate ..... 5. *R. tunguraguana*
4′. Leaves 4.5–7.0 mm long, 1.5–2.1 mm wide, margins serrulate or sub-entire ..... 2. *R. carolae*

1. Rhynchostegiopsis brasiliensis Broth., Akad. Wiss. Wien, Math.-Naturwiss. Kl., Denkschr. 83: 339. 1927. TYPE: Brazil. São Paulo: "In silvaticus prope Barra Mansa in districtu urbis Itapecirica, ad arbores, ca. 1000 m," *V. Schiffner 1454* (lectotype, here designated, H-BR!; isolectotype, BM!). Figure 4.

Plants small, pale yellow-green, growing in compact mats. *Stems* 10–30 × 1–2 mm, often fragile, creeping, somewhat complanate-foliate, branching frequently, irregularly pinnate; *rhizoids* infrequent. *Leaves* not much contorted wet or dry, spreading, somewhat falcate, evenly spaced, ovate, 1.0–1.5 × 0.4–0.6 mm, slightly concave below, plane above; apices short-acuminate; margins entire below, serrate to weakly serrulate above, sometimes almost entire; upper marginal cells mostly larger or only slightly larger than adjacent median cells; median leaf cells long-hexagonal, 80–137 × 12–17 μm; basal cells broader and shorter than median cells, 62–100 × 22–29 μm. *Asexual reproduction* unknown. *Perigonia* not seen. *Perichaetia* and *sporophytes* not seen.

Etymology. Named after Brazil, the country of the type collection.

Distribution. *Rhynchostegiopsis brasiliensis* is known only from southeastern Brazil (Fig. 2). Elevation 800–1000 m.

Ecology. On trees.

Discussion. This species is distinctive in its small size combined with its ovate leaves with short-acuminate apices. *Rhynchostegiopsis brasiliensis* is similar in size and habit to *R. flexuosa*, but the latter species is distinguished by its broader leaves with short-acuminate, straight leaf apices and shorter, more hexagonal median leaf cells. In leaf shape it resembles some of the Central Amer-

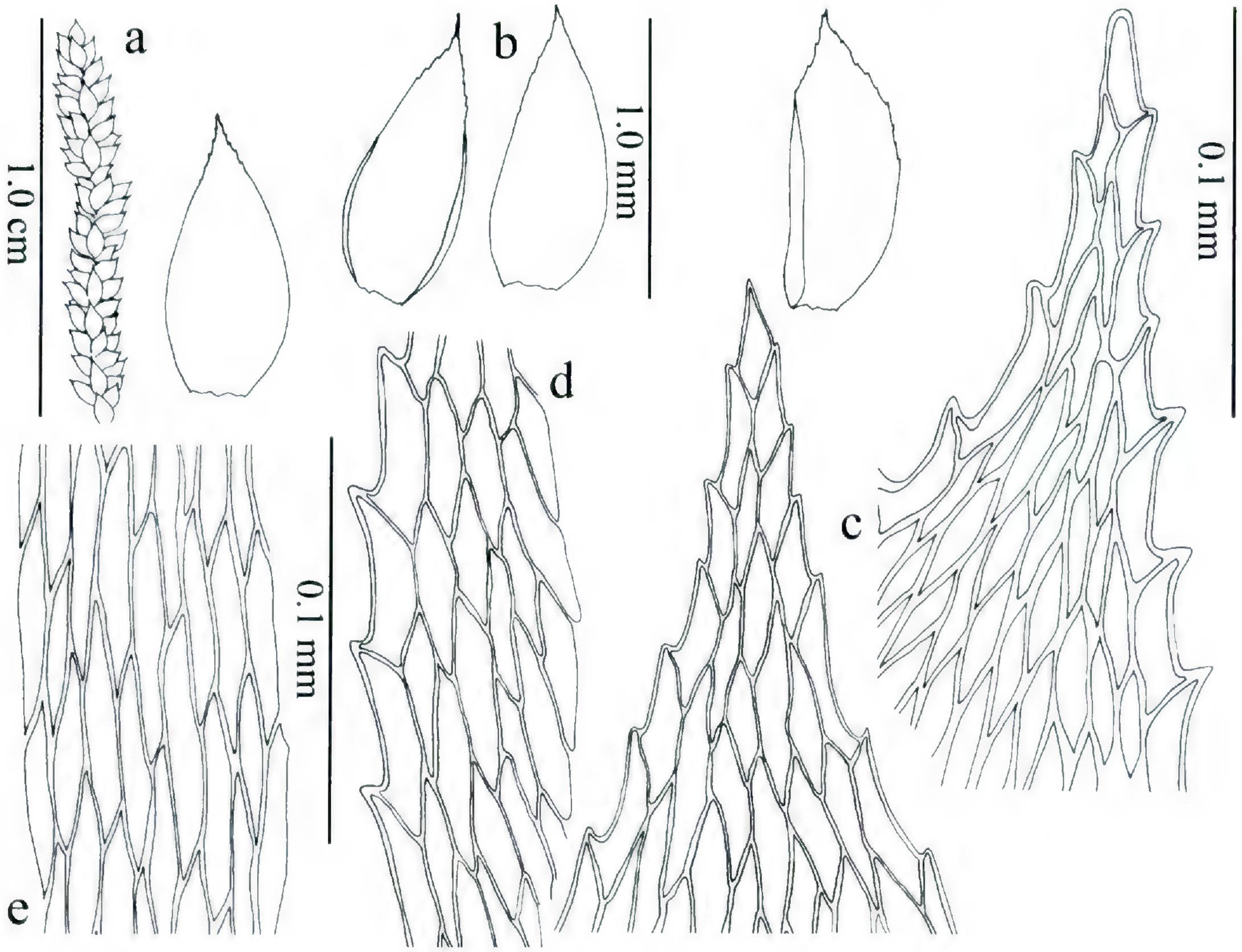


Figure 4. *Rhynchostegiopsis brasiliensis*. —a. Portion of stem. —b. A selection of leaves. —c. Leaf apices. —d. Leaf margin. —e. Median cells. (a, c–e: *Schiffner 1454*, BM, isolectotype; b: *Schiffner 1454, 679, 1122*, BM.)

ican specimens of *R. tunguraguana*, which can often have ovate leaves with short leaf apices, but it is easily distinguished by its much smaller size and wider, shorter leaf cells. Leaf margins in *R. brasiliensis* can vary from sharply serrate to almost entire, often on the same plant. This species may be mistaken for *Vesicularia* by its aspect but belongs in *Rhynchostegiopsis*, and not *Vesicularia*, because of the presence of two-celled axillary hairs and lack of pseudoparaphyllia on the stems. *Vesicularia* has foliose pseudoparaphyllia and axillary hairs that consist of a short brown basal cell and several elongate, hyaline upper cells. Gemmae, perichaetia/perigonia, and sporophytes are so far unknown in *R. brasiliensis*. Despite the lack of sporophyte material to confirm peristome characters, the position of this species in *Rhynchostegiopsis* is supported by the gametophyte features, especially stem anatomy and the lack of stem pseudoparaphyllia. *Rhynchostegiopsis brasiliensis* is currently known only from three localities around São Paulo, Brazil. In the bryophyte herbarium at MO, no further collections of this species were found among the unnamed material of *Vesicularia* or among the Hypnaceae or Hookeriaceae from Brazil.

Selected specimens examined. BRAZIL. **São Paulo:** In silvis ad "Brasso grande" in districtu urbis Itapecirica, ad arbores, ca. 1000 m, *V. Schiffner 1122* (syntypes, H-BR, BM); Prope Rio Grande ad "São Paulo Railway" ad arbores, 800 m, *V. Schiffner 679* (syntype, BM).

2. Rhynchostegiopsis carolae Crosby, Ann. Missouri Bot. Gard. 63: 373, f. 1–5. 1976 [1977]. TYPE: Costa Rica. San José: along Inter-American Highway, 13 km SE of El Empalme, 09°40′N, 83°51′W, 2600 m, 15 Mar. 1973, *M. R. Crosby & C. A. Crosby 5836* (holotype, MO!; isotypes, BA not seen, BM!, CR not seen, DUKE!, H not seen, MICH not seen, NICH not seen, NY!, US!). Figure 5.

Plants large, robust, pale green to yellow-green to golden with age, forming thin mats. *Stems* 50–100(–120) × 5–7 mm, creeping, complanate-foliate, infrequently sub-pinnately branched. *Leaves* slightly differentiated, dorsal leaves smaller than lateral leaves, evenly spaced, not crowded, not much contorted wet or dry, weakly complanate, erect-spreading, somewhat falcate, ovate-lanceolate, 4.5–6.0 × 1.5–2.1 mm, slightly concave below, flat above, apices long-acuminate; margins en-

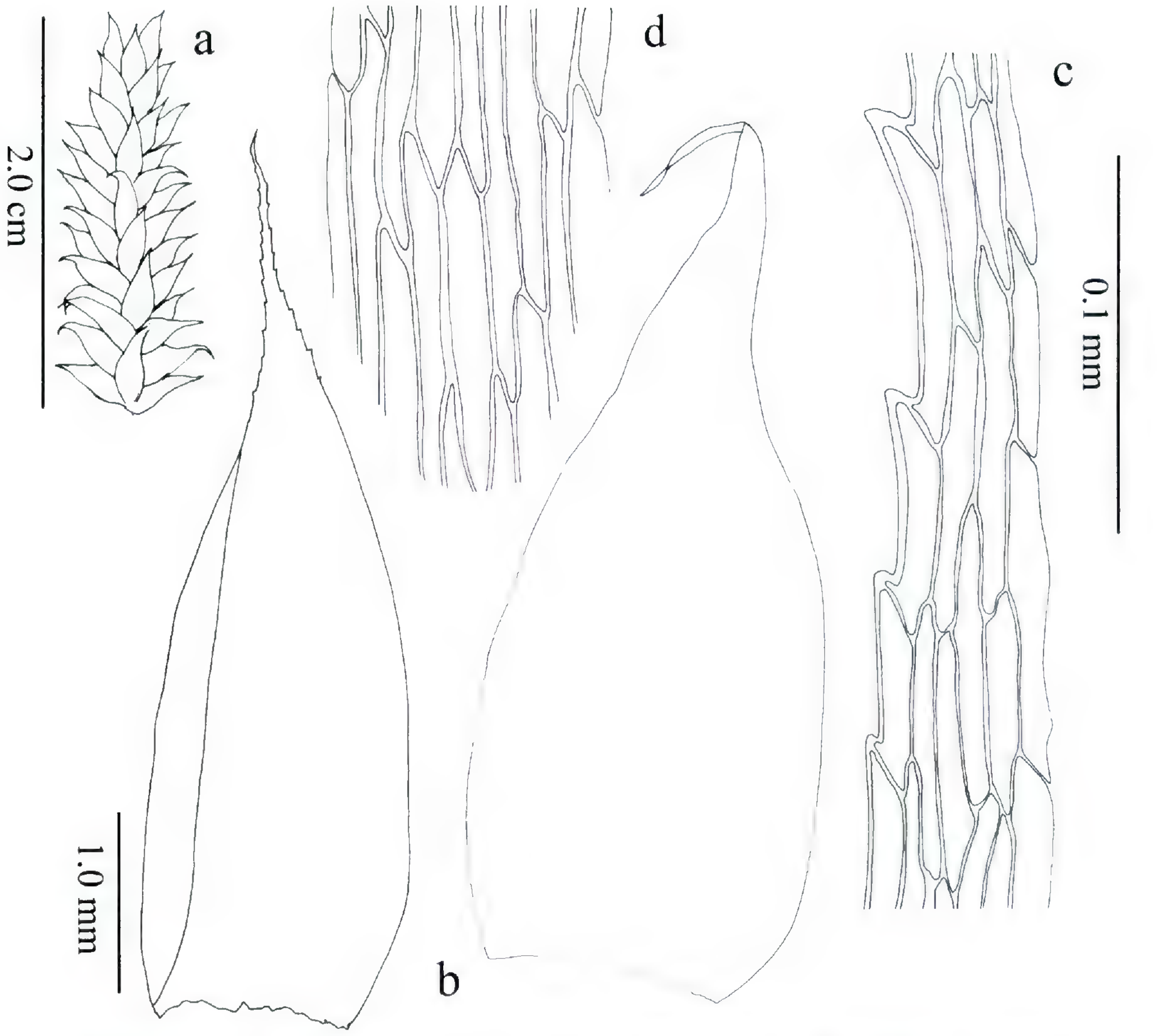


Figure 5. *Rhynchostegiopsis carolae*. —a. Portion of stem. —b. Leaves. —c. Leaf margin. —d. Median cells. (a–d: *Crosby 5836*, MO, holotype.)

tire below, weakly serrulate to sub-entire above; median cells long-hexagonal or long-linear, 125–290 × 11–27 μm; basal cells wider than but otherwise not much differentiated from median cells, 82–215 × 35–57 μm; upper marginal cells only weakly or not enlarged in comparison to median cells, 82–175 × 6–16 μm. *Asexual reproduction* unknown. *Perigonia* inconspicuous, leaves 0.8–1.6 mm, otherwise similar to perichaetial leaves. *Perichaetia* inconspicuous, leaves 1.5–2.3 mm, mostly ovate, gradually long-acuminate, apices entire or weakly serrulate. *Setae* 16–41 mm, smooth, dark red. *Capsules* inclined; *urn* cylindrical, 1.5–2.0 × to 1 mm; *annuli* not seen; *opercula* long-rostrate, 1.4–1.6 mm; *exostome teeth* 650–750 μm. *Calyptrae* to 2 mm. *Spores* 13–20 μm diam.

Etymology. Named after one of the collectors, Carol A. Crosby, daughter of L. E. Anderson.

Illustrations. Crosby (1976), figures 1–5.

Distribution. Costa Rica, Panama (Fig. 3c). Elevation 1400–2680 m.

Ecology. Moist and shaded terrestrial habitats such as road banks, ravines, and drainage ditches in montane forests.

Discussion. *Rhynchostegiopsis carolae* is distinct in its very large size and large leaves combined with the only slightly enlarged marginal cells in the upper leaf apex. In this species the leaves are not as crowded as those in *R. tunguraguana*, and they tend to be flattened along the stem. Large specimens of *Rhynchostegiopsis tunguraguana* can approach *R. carolae* in size, and leaf cell measurement can overlap; however, *Rhynchostegiopsis carolae* can be distinguished by its wider stems and larger, moderately spaced leaves with weakly serrulate to almost entire margins. It is presently known only from mid elevations (1400–2680 m) in central Costa Rica and from one locality in Panama.

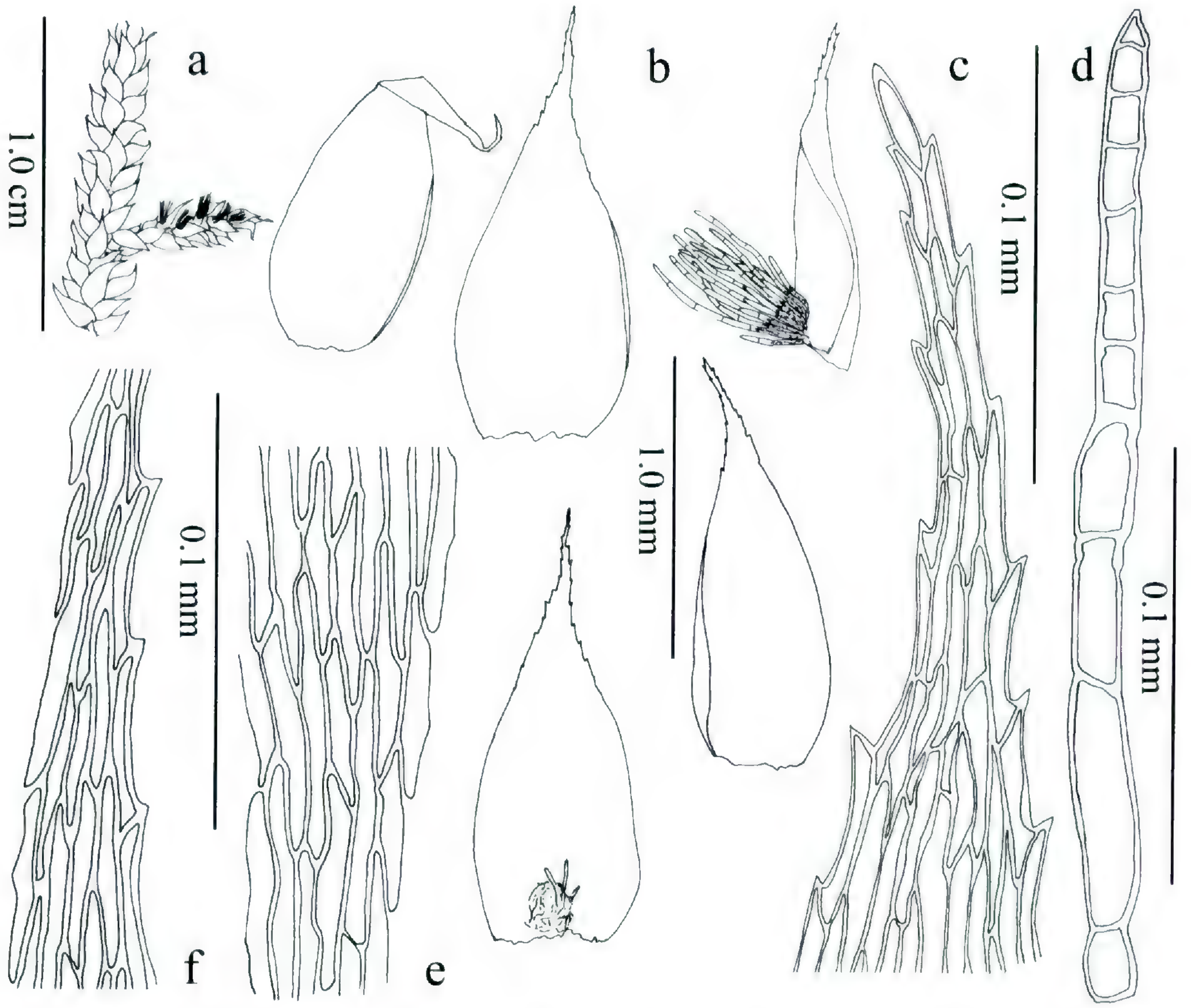


Figure 6. *Rhynchostegiopsis costaricensis.* —a. Portion of stem. —b. Leaves with and without leaf gemmae. —c. Leaf apex. —d. A leaf gemma. —e. Median cells. —f. Leaf margin. (a, c–e: *Morales 38*, US; b, f: *Crosby 9754*, MO.)

Selected specimens examined. COSTA RICA. **Cartago:** Dos Amigos, 2680 m, *Crosby & Crosby 5957* (MO). **Heredia:** Las Vueltas, 2100 m, *Crosby & Crosby 6008* (MO). **San José:** 17 km SE of El Empalme, 2650 m, *Crosby 10861* (MO). PANAMA. **Chiriquí:** Cerro Colorado, 1400 m, *Antonio 1432* (MO).

3. Rhynchostegiopsis costaricensis H. Rob. & D. G. Griffin, Phytologia 30: 281. 1975. TYPE: Costa Rica. Alajuela: Parque Nacional Volcán Poás, *Clusia* forest, E rim of crater, 2450 m, 3 Sep. 1973, *D. G. Griffin, III 92* (holotype, US!; isotype, FLAS not seen). Figure 6.

Plants small to medium, pale yellow to yellow-green; forming sparse mats. *Stems* 10–40 × 1–2 mm, often fragile, creeping, infrequently sub-pinnately branched. *Leaves* not much differentiated when dry, sometimes folded when wet, erect spreading, sometimes slightly complanate or falcate with leaf apices pointing to substrate, distant, slightly dimorphic, ovate to ovate-lanceolate, 1.0–2.4 × 0.4–0.9 mm, leaves concave below, plane above, short- to long-acuminate; margins entire below, serrulate or sub-entire above; upper marginal cells not or slightly larger than median cells, 50–90 × 6–11 μm; median cells long-linear to long-hexagonal, 90–155 × 8–15 μm; basal cells usually shorter and broader than median cells, 65–140 × 20–40 μm, sometimes differentiated in 2–4 rows or a triangular patch at base of leaves in association with leaf gemmae, differentiated cells thick-walled. *Asexual reproduction* frequent, leaf gemmae clustered at base of leaf on dorsal surface of upper stem leaves, prominent, dark reddish at base, hyaline, fragile above, 8–15 cells long. *Perigonia* not seen. *Perichaetia* lateral, leaves 0.7–1.2 mm, short- or long-acuminate from an ovate base, apices erect to spreading, margins serrulate to sub-entire. *Setae* 9–25 mm, smooth, reddish to dark red. *Capsules* inclined; *urn* ovoid-cylindrical, 1–2 × 1 mm; *annuli* not seen; *opercula* long-rostrate, 1.2 mm; *exostome teeth* 350–500 μm. *Calyptrae* 1.6–3.0 mm. *Spores* not seen.

Etymology. Named after Costa Rica, the country of the type collection.

Illustrations. Robinson and Griffin (1975).

Distribution. Honduras, Costa Rica (Fig. 3b). Elevation 2480–3130 m.

Ecology. On rotting logs, leaf litter, or on soil, less frequently as an epiphyte on trunks or twigs of living trees.

Discussion. This species is readily distinguished from all other species in *Rhynchostegiopsis* when leaf gemmae are present. The gemmae form "tufts" on the dorsal surface of the leaves that are visible with the naked eye. Although gemmae production is not species specific in this genus, their development from the dorsal leaf surface is unique to *R. costaricensis.* Gemmae have been observed at the leaf base in the type specimen of *R. tunguraguana* (*Spruce 1048,* NY), and in one other collection of the same species (*Ramírez 9039,* NY). In these specimens the gemmae were more loosely aggregated and less well developed than those in *R. costaricensis.* They were derived from the stem tissue at the very base of the leaf rather than on the dorsal leaf surface as in *R. costaricensis. Rhynchostegiopsis costaricensis* can be difficult to distinguish from other species in the absence of leaf gemmae. In size and habit it could be confused with the more robust forms of *R. flexuosa.* The straight, serrulate to sub-entire apices, marginal cells that are similar in size to adjacent median cells, and its more ovate to ovate-lanceolate leaves distinguish it from *R. flexuosa.* The long-acuminate, straight apices, serrulate leaf margins, marginal cells that are similar in size to adjacent median cells, and the narrow median cells are similar to some specimens of *R. tunguraguana.* These two species can be difficult to separate based on these characters alone. The leaves in *R. costaricensis* are more distant on the stems and are smaller with shorter, narrower median and upper marginal cells.

One feature that occurs sporadically in specimens of *R. costaricensis* is the presence of an area of differentiated cells at the base of the leaf. These differentiated cells are broader and longer with much thicker walls than the cells in other leaves in the equivalent position. These differentiated cells are found in 2–4 rows or as a triangular patch at the leaf base. This "patch" of cells is described in more detail by Robinson and Griffin (1975). These differentiated cells are found on leaves that have leaf gemmae, but they can often be found on leaves even if gemmae are not present. Sporophytes are not common in these specimens, but they have been found in species that also have leaf gemmae.

Selected specimens examined. HONDURAS. **Lempira:** Parque Nacional de Celaque, 2480 m, *Allen 11470* (MO). COSTA RICA. **Alajuela:** Parque Nacional Volcán Poás, 2520 m, *Araya et al. 81* (F, MO, NY, US). **Cartago:** just SE of summit of Cerro de la Muerte ridge, 3050 m, *Crosby 9789* (MO). **Heredia:** SW slopes of Volcán Barba, 2500 m, *Crosby 13128* (MO). **San José:** 10 km NW of summit at La Ascensión, 3130 m, *Crosby & Crosby 6117* (MO).

4. Rhynchostegiopsis flexuosa (Sull.) Müll. Hal., Nuovo Giorn. Bot. Ital. n.s. 4: 163. 1897. *Hypnum flexuosum* Sull., Proc. Amer. Acad. Arts 5: 288. 1861. *Leucomium flexuosum* (Sull.) Mitt., J. Linn. Soc., Bot. 12: 501. 1869. TYPE: Cuba. Locality not indicated, [1856B58] *C. H. Wright 112* (holotype, FH not seen; isotypes, BM!, H-BR!, NY!). Figure 7.

Rhychostegium cupressinum Besch., Mém. Soc. Sci. Nat. Cherbourg 16: 250. 1872. *Rhynchostegiopsis cupressina* (Besch.) Cardot, Rev. Bryol. 38: 101. 1911. TYPE: "Mexico. Cordova," *C. Sallé s.n.* (Sallé in herb. Decaisne) (holotype, PC not seen [in herb. of Decaisne]; isotypes, BM!, NY!).

Leucomium serratum Besch., J. Bot. (Morot) 8: 179. 1894. *Rhynchostegiopsis serrata* (Besch.) Broth., in Engl. & Prantl, Nat. Pflanzenfam. I(3): 948. 1907. TYPE: "Guadeloupe. Route du Gommier, sur les troncos pourris," *Ed. Marie 652* (lectotype, designated here, BM!; isolectotype, NY!). *Ed. Marie 664* (syntypes, BM!, NY!)

Vesicularia auricolor Müll. Hal., Bull. Herb. Boissier 5: 211. 1897. *Rhynchostegiopsis auricolor* (Müll. Hal.) Broth., in Engl. & Prantl, Nat. Pflanzenfam. (ed. 2) 11: 263. 1925. TYPE: "Guatemala. Alta Verapaz: Pansamala, 30 Jan. 1886," *H. v. Türckheim s.n.* (holotype, H-BR!; isotypes, BM!, NY!).

Plants small to medium, bright green to yellow-green, growing in compact or thin mats. *Stems* 10–30(–40) × 1–3 mm, often fragile, creeping, irregularly to sub-pinnately branched. *Leaves* spreading to erect-spreading, somewhat complanate-foliate, falcate, or falcate-secund, often with leaf apices pointing downward, usually crowded, sometimes distant, variously to not folded when dry or wet, sometimes slightly dimorphic, lanceolate to oblong-lanceolate, 1.0–2.5 × 0.2–0.6 mm, plane or slightly concave below, plane above; apices gradually long-acuminate, flexuose; margins entire below, sharply serrate to serrulate above, upper marginal cells larger than median cells, 100–150 × 18–25 μm; median cells long-linear to long-hexagonal, 100–130 × 12–20 μm; basal cells long-hexagonal, shorter than median cells, 62–82 × 15–17 μm. *Asexual reproduction* unknown. *Perigonia* lateral, leaves similar to perichaetial leaves. *Perichaetia* conspicuous, leaves lanceolate, 0.7–1.5 mm, apices long-acuminate, spreading, usually flexuose, margins serrate to sub-entire. *Setae* 10–30 mm, dark

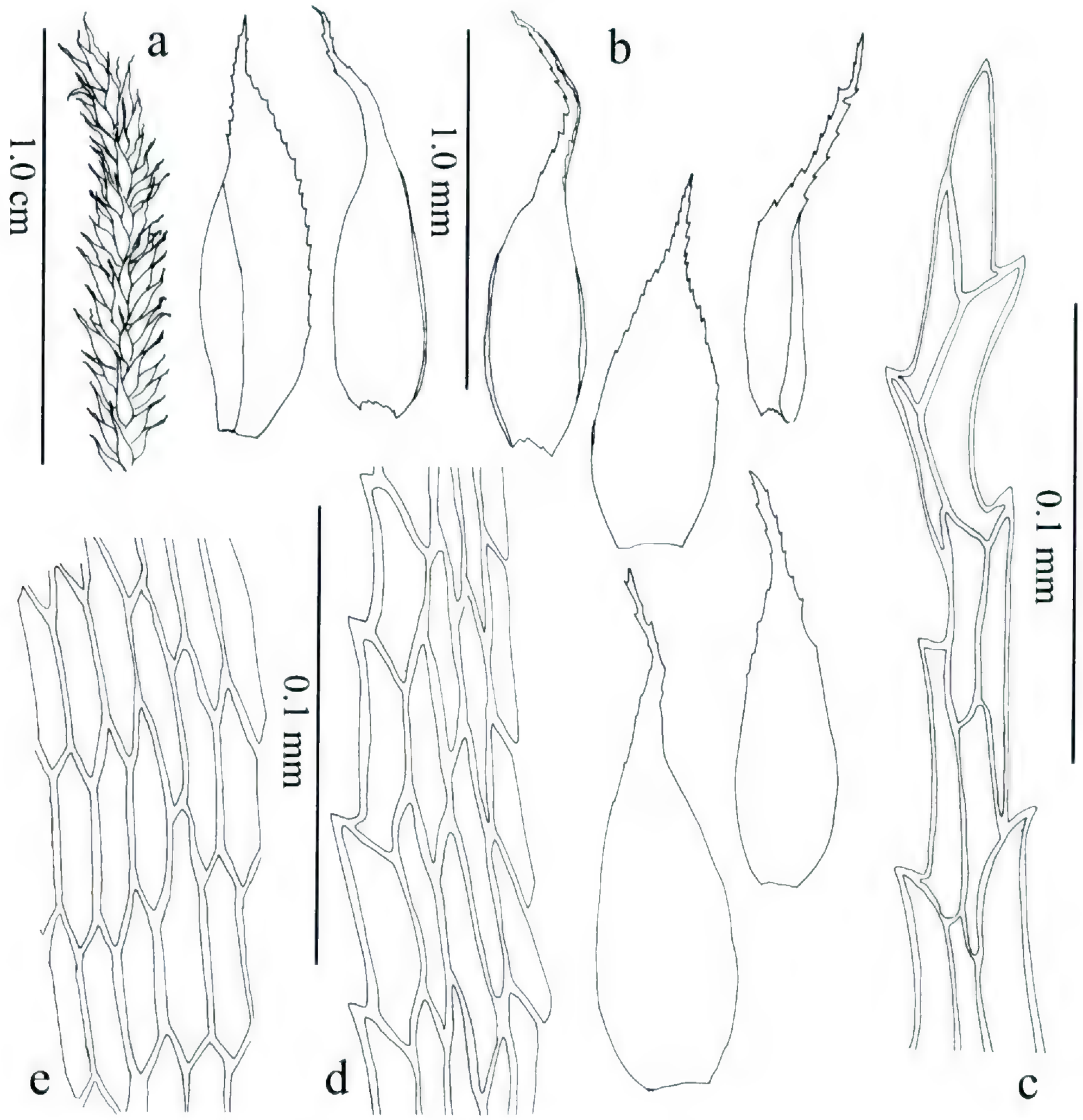


Figure 7. *Rhynchostegiopsis flexuosa*. —a. Portion of stem. —b. A selection of leaves. —c. Leaf apex. —d. Leaf margin. —e. Median cells. (a, c–e: *Wright 1121*, BM, isotype; b: *Wright 112*, BM, isotype, *Price 834*, MO, *Allen 11959*, MO, *Buck 7909*, NY, *Pringle 10481*, BM.)

reddish to orange, smooth or sometimes slightly roughened just below urn. *Capsules* inclined to pendent; *urn* cylindrical, 0.8–1.3 × 0.4–1 mm; *annuli* 2–5 layers of thin-walled cells, deciduous; *opercula* long-rostrate, 1.0–1.2 mm; *exostome teeth* 300–325 μm. *Calyptrae* 1.0–1.6 mm. *Spores* 11–15 μm diam.

Etymology. Derived from the Latin "*flexuosus*," meaning bent alternately in opposite directions in reference to the distinctive leaf apices.

Illustrations. Bartram (1949), figure 143; Crum and Bartram (1958), figure 629; Welch (1966), figures 288–292; Welch (1972), figures 261–267; Buck (1998), plate 37.

Distribution. Mexico, Central America, the Caribbean, and South America (Colombia) (Fig. 3a). Elevation 200–2850 m.

Ecology. On fallen, decaying logs, tree trunks or exposed roots of living trees, and less frequently on soil.

Discussion. The typical form of this species is distinct in its long-acuminate, often sharply serrate, flexuose leaf apices, and lanceolate or oblong-lanceolate leaves. The typical form has upper marginal cells that are wider and slightly longer than the adjacent median cells. These cells form what appears to be a border of enlarged cells along the upper leaf margins. *Rhynchostegiopsis flexuosa* is commonly found with sporophytes, even though it is dioicous. The typical form of *R. flexuosa* is most likely to be confused with *Leucomium*. It is similar in habit to *Leucomium strumosum*, but the serrate margins of *R. flexuosa* distinguish these species. Both species are similar in habit to some members

of the Hypnaceae, such as *Vesicularia*, but the absence of pseudoparaphyllia, undifferentiated alar cells, the two-celled axillary hairs, and distinct stem anatomy are characters that align them instead with the Hookeriales and/or the Leucomiaceae.

Within the genus *Rhynchostegiopsis* it is difficult to confuse the typical form of *R. flexuosa* with any of the other four species because of its small, narrow, lanceolate leaves; sharply serrate leaf margins; and long-acuminate, flexuose leaf apices. However, *R. flexuosa* is the most common and variable of the species in *Rhynchostegiopsis*. Variability is found in plant size, leaf size, leaf shape, apex length, the size of serrations, and the degree to which the apex is flexed. A rather confusing degree of variation is seen in a group of Central American specimens, mainly from Honduras and Belize. These plants have various combinations of the following characters that are different than the typical form of *R. flexuosa*: almost straight apices; short apices; only slightly serrate leaf margins; marginal cells similar in size to the adjacent median cells; or larger more ovate leaves. These specimens are often larger than the more typical forms of *R. flexuosa*, instead being more similar in size to plants previously known as *R. lutescens* (= *R. tunguraguana*). It is difficult to break down character variation seen in these plants in any informative way, since specimens that differ in one character can be typical for all others. For example, a specimen of *R. flexuosa* may have small, narrow, lanceolate leaves, marginal cells that are well developed, and serrate leaf margins, but with apices that are either non-flexuose or approach the short-acuminate form.

In this work, *Rhynchostegiopsis auricolor*, *R. cupressina*, and *R. serrata* remain in synonymy with *R. flexuosa*. The species *Rhynchostegiopsis auricolor*, first described as *Vesicularia auricolor*, was confirmed as being *R. flexuosa*. Plants from Mexico, previously named *Rhynchostegium cupressina* (*Rhynchostegiopsis cupressinum*), including the type specimen (*Sallé s.n.*, BM, NY) and a collection made by Pringle (*Pringle 10481* from BM, F, MO, NY, US), have slightly more ovate leaves with shorter, non-flexuose apices than the typical form of *R. flexuosa* (see leaf variation in Fig. 7b). However, their median cells, marginal cells, and serrate leaf margins are typical of *R. flexuosa*, supporting the synonymy of *R. cupressina* with *R. flexuosa*. *Rhynchostegiopsis serrata*, first described as *Leucomium serratum*, was differentiated from *R. flexuosa* by its autoicous sexuality and short seta length (Brotherus, 1907). The setae of *R. flexuosa* are characteristically long, but a degree of variation in the length of setae was observed, particularly in specimens that were depauperate. Type material of *Leucomium serratum* was sterile, thus sexual condition could not be determined. In all other characters these plants were identical to *R. flexuosa*. Welch (1972) considered material of *R. serrata* from Guadeloupe, *Marie 652, 664* (BM, NY), and St. Vincent, *Morton 5506* (US), to be distinct from *R. flexuosa* because of their smaller size, serrate rather than sharply serrate upper leaf margins, and less flexuose leaf apices. However, none of these characters support *R. serrata* as a separate taxon.

Selected specimens examined. MEXICO. **Chiapas:** Pueblo Nueva, ca. 2000 m, *Sharp et al. 4209* (F, MO, US). **Guerrero:** 31 km N of El Paraiso, 2000 m, *Thomas & Contreras 3765* (NY). **Hidalgo:** Zacualtipan, 2100 m, *Sharp et al. 1872* (F). **Jalisco:** slopes of La Ferreria, *Crum 1068* (F, NY). **Oaxaca:** 15 mi. above Valle Nacional, *Sharp et al. 4520* (MO, F). **San Luis Potosí:** W of Xilitila, ca. 1200 m, *Sharp 5966* (US). **Veracruz:** Agua de la Calabaza, 1800 m, *Juarez 1046* (MO). BELIZE. **Cayo:** Doyle's Delight, 1098 m, *Allen 15122* (MO). **Toledo:** Southern Maya Mountains, 1000 m, *Allen 15464* (MO). GUATEMALA. **Alta Verapaz:** Samac, ca. 1400 m, *Johnson 791* (NY, US). **Baja Verapaz:** along dirt road 4 mi. NE of Purulhá, 1500 m, *Croat 41305* (MO). **Chimaltenango:** slopes of Volcán Acatenango, 2400–2700 m, *Standley 61818* (F, MO). **Huehuetenango:** Sierra de los Cuchumatanes, 1500 m, *Steyermark 20056* (MO). **Quetzaltenango:** Volcán de Zumil, 2850 m, *Standley 64444* (MO). **San Marcos:** El Porvenir near Volcán Tajumulco, 1100 m, *Sharp 5372* (MO). **Suchitepequez:** Volcán Santa Clara, 1250–2650 m, *Steyermark 46711* (F). **Zacapa:** Río Sito Nuevo, 1500–1800 m, *Steyermark 13226* (F, NY). EL SALVADOR. **Santa Ana:** trail to the top of Monte Cristo, ca. 2300 m, *Watson ES-0022* (MO). HONDURAS. **Atlántida:** Lancetilla Valley, 200–600 m, *Standley 51633* (BM, F, NY, US). **Comayagua:** San Juanillo, 1700–2000 m, *Liesner 26810* (MO). **Copán:** El Zapote, 900B1000 m, *Allen 17768* (MO). **Cortés:** summit of Cerro Cantiles, 1600–2000 m, *Allen 14198* (MO). **Francisco Morazán:** Cerro de Uyuca, 1600 m, *Standley 4805* (F, MO). **La Paz:** Las Trances, 2000–2100 m, *Liesner 26479* (MO, NY). **Lempira:** Montana de Claque, 2040 m, *Allen 11263* (MO, NY). **Olancho:** La Muralla Biological Reserve, 1115 m, *Allen 12497* (MO). **Santa Bárbara:** E slope of Mt. Santa Barbara, 2220–2640 m, *Allen 11632* (MO). **Yoro:** Cordillera Nombre de Dios, ca. 300–400 m, *Allen 133615* (MO). NICARAGUA. **Jinotega:** N slope of Volcán Yali, 1200–1400 m, *Stevens & Grijalva 15265* (MO). COSTA RICA. **Alajuela:** Viento Fresco, 1600–1900 m, *Standley & Torres 47877* (US). **Cartago:** El Muñeco, 1100 m, *Standley 33697* (NY, US). **Guanacaste:** Parque Nacional de Guanacaste, 700 m, *Chávez 73* (MO). **Heredia:** Porrosati Area, 2250 m, *Crosby & Crosby 6021* (MO). **San José:** San Maria de Data, 1800 m, *Standley 42934b* (US). PANAMA. **Bocas del Toro:** on hwy. to Chiriquí Grande, 250–300 m, *Allen 5542* (MO). **Chiriquí:** Volcán Barú, 1750–1900 m, *Almeda 6197* (MO). **Darién:** Cana, 800–1200 m, *Allen 8843* (MO, NY). CUBA. Locality not indicated, *Wright 112 & 113* (BM, NY). **Potosí:** Monte Toro, *Wright 173* (MO). **San Juan:** San Juan, *Hioram 12539* (MO). JAMAICA. **St. Thomas:** Cuna Cuna

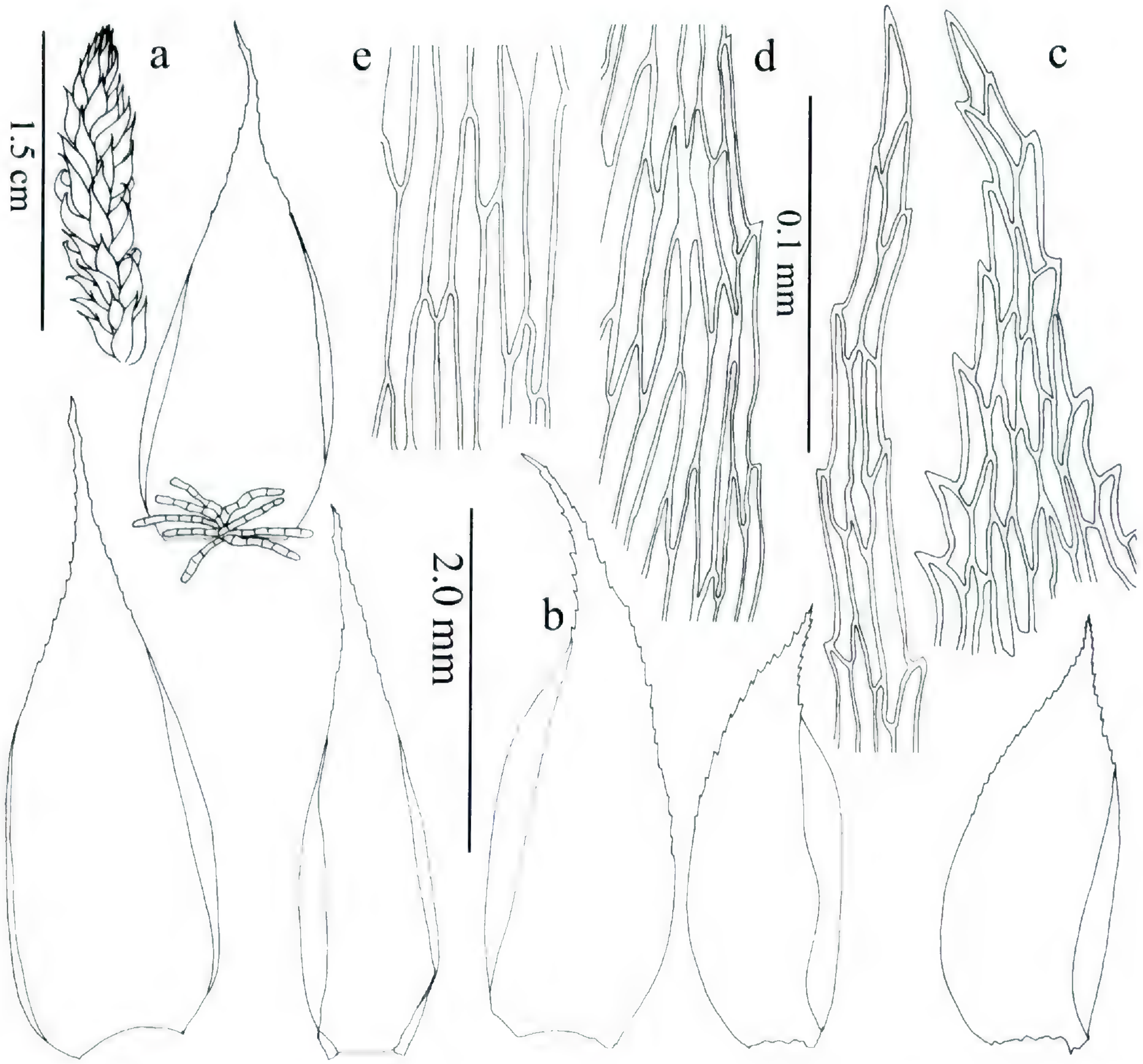


Figure 8. *Rhynchostegiopsis tunguraguana*. —a. Portion of stem. —b. Leaves. —c. Leaf apices. —d. Leaf margin. —e. Median cells. (a, c–e: *Spruce 1048*, NY, holotype; b: *Lewis 83–710*, LPB, *Britton 1142*, NY, *Spruce 1048*, NY, holotype.)

Trail, *Maxon & Killip 163* (BM, F, NY, US). DOMINICAN REPUBLIC. **La Vega:** Constanza, ca. 1500 m, *Buck 7909* (NY). TRINIDAD. Locality not indicated, *Cruger s.n.* (BM, NY). PUERTO RICO. **Las Piedras:** El Toro, 400–550 m, *Price 834* (MO). GUADELOUPE. Locality not indicated, *Hooker 190* (NY); Route du Gommier, sur les troncos pourris, *Ed. Marie 664* (syntypes of *L. serratum*, BM!, NY!). DOMINICA. Morne Micotrin, ca. 1000 m, *Elliot 22* (BM). ST. VINCENT. Cumberland River, 400–600 m, *Morton 5506* (US). COLOMBIA. **Meta:** Villavicencio, 765–1080 m, *Steere 7671* (MO, NY).

5. Rhynchostegiopsis tunguaraguana (Mitt.) Broth., in Engl. & Prantl, Nat. Pflanzenfam. I(3): 1235. 1909. *Stereodon tunguraguanus* Mitt., J. Linn. Soc., Bot. 12: 534. 1869. TYPE: Ecuador. "Andes Quitensis, in monte Tunguragua supra pagum Puela, 6000 ped," *R. Spruce 1048* (holotype, NY!; isotypes, BM!, H-BR!). Figure 8.

Rhynchostegiopsis complanata Müll. Hal., Nuovo Giorn. Bot. Ital. n.s. 4: 163. 1897. No specimen cited. TYPE: Bolivia. Based on: "Cochabamba: prope Choquecamata, June 1889," *P. Germain 1147* (lectotype, here designated, NY!; isolectotype, H-BR!).

Rhynchostegiopsis lutescens E. Britton, in Broth., in Engl. & Prantl, Nat. Pflanzenfam. (ed. 2) 11: 263. 1925. No specimen cited. TYPE: Jamaica. Based on: "St. Andrew: Slopes of Sir John and summit, Blue Mountains, 5 Oct. 1908," *E. G. Britton 1142* (holotype, NY!; isotypes, BM!, H-BR!); see Crum & Bartram (1958).

Rhynchostegiopsis planifolia H. A. Crum & E. B. Bartram, Bull. Inst. Jamaica 8: 55. 1958. Nom. illeg. incl. sp. prior. TYPE: "Jamaica. St. Thomas: Terrestrial, moist bank in montane rain forest, 6000 ft., Blue Mountain Peak track, 16 Dec. 1951," *R. G. Robbins 89* (holotype, FH not seen; isotype, US!).

Plants medium to large, robust, pale yellow-green to golden; forming dense to sparse mats.

Stems 35–70(–100) × 2–5 mm, stems sometimes fragile, creeping or spreading, infrequently subpinnately branched. *Leaves* slightly folded when dry, folded when wet, complanate to spreading, often falcate with leaf apices pointing to substrate, usually crowded, slightly dimorphic, ovate to ovate-lanceolate, 1.7–4.6 × 0.7–1.6 mm, leaves concave below, plane above; apices short- or long-acuminate, sometimes flexuose; margins entire below, serrate to serrulate above; upper marginal cells, not or slightly larger than median cells, 50–200 × 6–20 μm; median cells long-linear, 52–288 × 8–37 μm; basal cells usually shorter and broader than median cells, 52–157 × 17–40 μm. *Asexual reproduction* infrequent, gemmae at base of leaf, not prominent, slightly orange colored at base, hyaline above, up to 8 cells long. *Perigonia* lateral, inconspicuous. *Perichaetia* lateral, conspicuous, leaves 0.7–1.9 mm, short- or long-acuminate from an ovate base, apices serrulate to sub-entire. *Setae* 20–25 mm, smooth reddish to dark red. *Capsules* inclined; *urn* ovoid-cylindrical, 2–2.5 × 0.5–1.4 mm; *annuli* not seen; *opercula* long-rostrate, 1.5 mm; *exostome teeth* 500–600 μm. *Calyptra* 3.8 mm. *Spores* 12–19 μm diam.

Etymology. Derived from its collection locality in Ecuador.

Illustrations. Crum (1994), figure 630; Churchill and Linares (1995), figure 119; Buck (1998), plate 36.

Distribution. Mexico, Central America, the Caribbean, and South America (Fig. 2). Elevation 600–3450 m.

Ecology. On roots, trunks, or branches of living trees, rotting logs, leaf litter, and moist shaded terrestrial habitats such as road banks, ravines, and drainage ditches.

Discussion. This species is best distinguished by its habit with complanate, leafy stems, and leaves with apices that often point downward. The leaves of *R. tunguraguana* are ovate to ovate-lanceolate with either gradually long- or short-acuminate apices. Larger specimens of *R. tunguraguana* may be confused with *R. carolae*, but they can be separated by their leaf apices, more crowded leaves, length of median cells, and the size of marginal cells. Only one calyptra of *R. tunguraguana* was seen during this study, *Churchill & Sastre de Jesus 12999* (NY), and it was longer than the calyptrae seen in specimens of *R. carolae*. The calyptra of *R. tunguraguana* was 3.8 mm, while those seen in *R. carolae* were up to 2 mm in length. *Rhynchostegiopsis tunguraguana* has been found with gemmae at the base of the leaf, a character previously thought to be found exclusively in *R. costaricensis*. The gemmae in *R. tunguraguana* are more loosely arranged, much shorter, and less well developed than those seen in *R. costaricensis*; see discussion under *R. costaricensis* (Figs. 6, 8).

Examination of plants from the Caribbean, Central America, and South America revealed a pattern of continuous character variation in *R. lutescens*, *R. complanata*, and *R tunguraguana*. Plants from Jamaica, the Dominican Republic, and Central America, previously known as *R. lutescens*, often have shorter, more sharply serrate leaf apices than the South American plants. However, they are similar in all other characters to *R. tunguraguana*. The name *R. lutescens* had been previously placed under the synonymy of *R. tunguraguana* (Crum, 1994; Buck, 1998), which is upheld in this work. Plants from Bolivia, earlier identified as *R. complanata*, have very long-acuminate, sometimes flexuose leaf apices, and marginal cells that form a border. Their long-acuminate, sometimes flexuose leaf margins are similar to those of *R. flexuosa*, but their much larger size and larger leaf cells distinguish them from this species. In all other characters, *R. complanata* falls within the variation of *R. tunguraguana* and is maintained in synonymy of it.

There has been some confusion over the validity and use of the names *Rhynchostegiopsis lutescens* and *R. planifolia* (Welch, 1971). *Rhynchostegiopsis lutescens* was published with a minimal description by E. G. Britton in Brotherus (1925). This name was incorrectly cited as being a nomen nudum by Crum and Bartram (1958), who placed it in synonymy of their newly described *R. planifolia*. In the herbarium at The New York Botanical Garden, the specimen *Britton 1142* ascribed to *R. lutescens* includes a lengthy description of this species and illustrations of the plant by E. G. Britton, presumably intended for publication. Although no type was cited for this species in the original publication (Brotherus, 1925), this specimen has been considered to be the type based on the presence of the unpublished manuscript with the herbarium sheet (Crum & Bartram, 1958). The description of *R. planifolia* by Crum and Bartram (1958) was based on some of the same specimens that had been examined by E. G. Britton, including the putative type (*Britton 1142*, NY) and two other specimens from Jamaica (*Nichols 179 & 184*, both NY), which were then included in the protologue of *R. planifolia*. The description of *R. planifolia* incorporated parts taken from the unpublished manuscript of E. Britton (Crum & Bartram, 1958). Although *R. planifolia* was based on a different type specimen (*Robbins 89*, FH, US) it is an illegitimate name because

it included the valid prior name *R. lutescens*. Welch (1971) used the name *R. planifolia* based on the assumption that *R. lutescens* was not validly published, because it lacked a Latin diagnosis. However, a Latin diagnosis was not required at the time of publication of the original article. Wijk et al. (1967) correctly treated the name *R. planifolia* as an illegal name.

Selected specimens examined. MEXICO. **Oaxaca:** S of Valle Nacional, ca. 3000 m, *Whittemore 1889A* (MO). BELIZE. **Toledo:** Columbia River Forest Reserve, 600–700 m, *Allen 18539* (MO). COSTA RICA. **San José:** Las Nubes, *Wendland s.n.* (BM, NY). HONDURAS. **Francisco Morazán:** El Parque Nacional la Tigra, 2000 m, *Allen 12368* (MO). **Lempira:** Montaña de Celaque, 2250 m, *Allen 12219* (MO). **Ocotepeque:** Nueva Ocotepeque, 2000–2180 m, *Allen 14452* (MO). **Olancho:** Montaña Babilonia, *Allen 12713* (MO). JAMAICA. **St. Andrew:** Sir John Peak, *Nichols 179 & 184* (NY). **St. Thomas:** Blue Mountains, Morce's Gap, *Nichols 37* (BM). DOMINICAN REPUBLIC. **La Vega:** La Nevera, 2100 m, *Buck 5395* (NY). VENEZUELA. **Trujillo:** Páramo de Guaramacal, 2800 m, *Dorr et al. 5034* (NY). COLOMBIA. **Antioquia:** Alto de Boguerón, 2800–3000 m, *Churchill et al. 14255* (MO). **Cauca:** Cerro Munchique, 2300–2555 m, *Churchill & Betancur 18150* (MO, NY). **Magdalena:** Río Buritaca, 2700 m, *Cleef et al. 302* (MO). **Nariño:** Alto Zapallurco, 3250 m, *Ramírez & Salas 10863* (MO). **Putumayo:** Reserva Natural La Rejoya, 2750 m, *Ramírez 10205* (MO). ECUADOR. **Carchi:** La Estrellita, 3300 m, *Dorr & Barnett 6149* (NY). **Chimborazo:** Puela, 1850 m, *Spruce 1048* (BM, NY). **Imbabura:** Lago San Marcos, 3450 m, *Cazalet & Pennington 52* (NY). **Loja:** Cajanuma, 2800 m, *Laegaard & Luzano 18518* (MO). **Napo:** Guagra Urcu, 2800 m, *Holm-Nielsen et al. 27339* (MO). **Sucumbios:** San Gabriel, 21501–2615 m, *Steere 9133* (NY). BOLIVIA. **Cochabamba:** Río Tocorani, 2200 m, *Herzog 4025* (BM, MO, NY). **La Paz:** Chusipata, ca. 2950 m, *Lewis 83–710* (LPB).

Literature Cited

Allen, B. H. 1987. A revision of the genus *Leucomium* (Leucomiaceae). Mem. New York Bot. Gard. 45: 661–677.

——— & M. R. Crosby. 1986. A revision of the genera *Pilotrichidium* and *Diploneuron* (Musci: Hookeriaceae). J. Hattori Bot. Lab. 61: 45–64.

———, ——— & R. E. Magill. 1985a. A review of the genus *Stenodictyon* (Musci). Lindbergia 11: 149–156.

———, ——— & ———. 1985b. Observations on the peristome and systematic position of *Tetrastichium fontanum* (Musci). J. Bryol 13: 515–522.

Anderson, L. E. 1954. Hoyer's solution as a rapid permanent mounting medium for bryophytes. Bryologist 57: 242–244.

Bartram, E. B. 1949. Mosses of Guatemala. Fieldiana, Bot. 25: 1–442.

Brotherus, V. F. 1907–1909. Musci (Laubmoose). II Pleurocarpi. *In* A. Engler & K. Prantl (editors), Nat. Pflanzenfam. I(3): 865–1008 (1907), 1009–1152 (1908), 1153–1173 (1909). W. Engelmann, Leipzig, Germany.

———. 1925. Musci (Laubmoose) 2. Hälfte. *In* A. Engler & K. Prantl (editors), Nat. Pflanzenfam. (ed. 2) 11: i–iv, 1–542. W. Engelmann, Leipzig, Germany.

Buck, W. R. 1987. Taxonomic and nomenclatural rearrangement in the Hookeriales with notes on West Indian taxa. Brittonia 39: 210–224.

———. 1988. Another view of familial delimitation in the Hookeriales. J. Hattori Bot. Lab. 64: 29–36.

———. 1990. Biogeography of the Greater Antillean mosses. Trop. Bryol. 2: 35–48.

———. 1991. The basis for familial classification of pleurocarpous mosses. Adv. Bryol. 4: 169–185.

———. 1992. A revision of *Philophyllum* (Hookeriales). Bryologist 95: 334–337.

———. 1998. The pleurocarpous mosses of the West Indies. Mem. New York Bot. Gard. 82: 1–400.

——— & D. H. Vitt. 1986. Suggestions for a new familial classification of pleurocarpous mosses. Taxon 35: 21–60.

Churchill, S. P. 1989. Transfer of *Lepidopilum virens* Cardot to *Tetrastichum* (Leucomiaceae). J. Bryol. 15: 537–541.

——— & E. L. Linares. 1995. Prodromus Bryologiae Novo-Granatensis. Bibliot. José Jerónimo Triana 12: 455–921.

Crosby, M. R. 1974. Toward a revised classification of the Hookeriaceae (Musci). J. Hattori Bot. Lab. 38: 129–141.

———. 1976. *Rhynchostegiopsis carolae* (Musci: Hookeriaceae): A new species from Costa Rica. Ann. Missouri Bot. Gard. 63: 373–375.

Crum, H. A. 1994. Leucomiaceae. *In* A. J. Sharp, H. A. Crum & P. M. Eckel (editors), The Moss Flora of Mexico. Mem. New York Bot. Gard. 69(2): 830–834.

——— & E. B. Bartram. 1958. A survey of the moss flora of Jamaica. Bull. Inst. Jamaica, Sci. Ser. 8: 1–90.

——— & W. C. Steere. 1957. The mosses of Porto Rico and the Virgin Islands. Sci. Surv. Porto Rico & Virgin Islands 7: 396–599.

Dixon, H. N. 1932. Classification of Mosses. Pp. 397–412 *in* F. Verdoorn (editor), Manual of Bryology. Nijhoff, The Hague.

ESRI. 1998. ArcView GIS. Environmental Systems Research Institute, Redlands, California.

Fleischer, M. 1906–1908 [1908]. Die Musci der Flora von Buitenzorg III. 645–1103. Leiden, Netherlands.

Hattaway, R. A. 1984. A Monograph of the Ptychomniaceae (Bryopsida). Ph.D Thesis, The Pennsylvania State University, University Park, Pennsylvania.

Hedenäs, L. 1995. Higher taxonomic level relationships among diplolepidous pleurocarpous mosses—A cladistic overview. J. Bryol. 18: 723–781.

———. 1996a. A cladistic overview of the "Hookeriales." Lindbergia 21: 107–138.

———. 1996b. A cladistic evaluation of relationships between the Hookeriales, the Sematophyllaceae and some other taxa. Lindbergia 21: 49–82.

Hutchinson, J. 1959. The Families of Flowering Plants. Ed. 2, 2nd Vol. Oxford Univ. Press, Oxford, England.

Miller, H. A. 1971. An overview of the Hookeriales. Phytologia 21: 243–252.

Müller, C. 1897a. Bryologia Guatamalensis. Bull. Herb. Boissier 5: 171–220.

———. 1897b. Prodromous bryologiae Bolivianae. Nuovo Giorn. Bot. Ital. n.s. 4: 110–172.

Robinson, H. 1971. A revised classification for the orders and families of mosses. Phytologia 21: 289–293.

———. 1975. The mosses of the Juan Fernandez Islands. Smithsonian Contr. Bot. 27: iii–iv, 1–88.

——— & D. Griffin III. 1975. A new species of *Rhynchostegiopsis* from Costa Rica. Phytologia 30: 281–283.

Taylor, E. C. 1962. The Philibert peristome articles. An abridged translation. Bryologist 65: 175–212.

Vitt, D. H. 1984. Classification of the Bryopsida. Pp. 696–759 *in* R. M. Schuster (editor), New Manual of Bryology, Vol. 2. Hattori Botanical Laboratory, Nichinan, Japan.

Welch, W. H. 1966. The Hookeriaceae of Mexico. Bryologist 69: 1–68.

———. 1971. The Hookeriaceae of Jamaica, Hispaniola and Puerto Rico. Bryologist 74: 77–130.

———. 1972. Hookeriaceae of the Lesser Antilles. Bryologist 75: 404–455.

Whittemore, A. & B. Allen. 1989. The systematic position of *Adelothecium* Mitt. and the familial classification of the Hookeriales (Musci). Bryologist 92: 261–272.

Wijk, R. van der, W. D. Margadant & P. A. Florschütz. 1967. Index Muscorum 4 (P–S). Regnum Veg. 48: 1–604.

Appendix 1

List of species

1. *Rhynchostegiopsis brasiliensis* Broth.
2. *Rhynchostegiopsis carolae* Crosby
3. *Rhynchostegiopsis costaricensis* H. Rob. & D. G. Griffin
4. *Rhynchostegiopsis flexuosa* (Sull.) Müll. Hal.
5. *Rhynchostegiopsis tunguraguana* (Mitt.) Broth.

Index to specimens examined

Specimens examined are listed below alphabetically by collector. Records include collection number and herbarium locations for each specimen. The species is indicated by a number in parentheses corresponding to the number in the List of Species.

Acuña 450 (DUKE) (4), 452 (DUKE, NY) (4); Allard 18624 (US) (4), 18753 (NY) (4); Allen 5542 (MO) (4), 5820 (MO) (4), 8843 (MO, NY) (4), 8869 (MO) (4), 11041 (MO) (4), 11043 (MO) (4), 11051 (MO) (4), 11054 (MO) (5), 11061 (MO) (5), 11067 (MO) (5), 11068 (DUKE, MO, NY) (5), 11072 (MO) (4), 11081 (MO) (5), 11209 (MO) (4), 11263 (MO, NY) (4), 11267 (MO) (4), 11309 (MO) (4), 11315 (MO) (4), 11373 (MO) (4), 11374 (MO) (4), 11377 (MO) (4), 11392 (MO) (4), 11404 (MO) (4), 11421 (MO) (4), 11470 (MO) (3), 11525 (MO) (4), 11526 (MO) (4), 11615 (MO) (4), 11632 (MO) (4), 11923 (DUKE, MO) (4), 11924 (MO) (4), 11925 (MO) (4), 11959 (MO) (4), 12028 (MO) (4), 12040 (MO) (5), 12042 (MO) (4), 12060 (MO) (5), 12064 (MO) (5), 12112 (DUKE, MO) (4), 12218 (MO) (4), 12219 (MO) (5), 12259 (MO) (4), 12285 (MO) (5), 12318 (MO) (5), 12325 (MO) (4), 12343 (MO, NY) (4), 12363 (MO) (4), 12368 (MO) (5), 12369 (MO) (4), 12497 (MO) (4), 12567 (MO) (4), 12571 (MO) (4), 12588 (MO) (4), 12670 (MO) (4), 12713 (MO) (5), 12855 (MO) (4), 12869 (MO) (4), 13615 (MO) (4), 13618 (MO) (4), 13775 (MO) (4), 13791 (MO) (4), 13792 (MO) (4), 13816 (MO) (4), 13901 (MO) (4), 13902 (MO) (4), 14198 (MO) (4), 14199 (MO) (4), 14206 (DUKE, MO) (4), 14326 (MO) (4), 14399 (MO) (4), 14452 (MO) (5), 14481 (MO) (5), 14504 (MO) (5), 14547 (MO) (5), 15101 (MO) (4), 15122 (MO) (4), 15126 (MO) (4), 15139 (DUKE, MO) (4), 15158 (MO) (4), 15334 (MO) (4), 15353 (MO) (4), 15395 (MO) (4), 15420 (MO) (4), 15464 (MO) (4), 15479 (MO) (4), 17329 (MO) (4), 17580 (MO) (4), 17581 (MO) (4), 17730 (MO) (4), 17768 (MO) (4), 17760 (MO) (4), 17772 (MO) (4), 18527 (MO) (4), 18539 (MO) (5), 18597 (MO) (4), 18603 (MO) (4), 18605 (MO) (4), 18651 (MO) (4), 18710 (MO) (4), 18986 (MO) (4), 19006 (MO) (4); Almeda & McPherson 6197 (MO) (4); Antonio 1432 (MO) (2); Araya & Griffin 81 (DUKE, F, MO, NY, US) (3).

Barnes & Land 572 (H, NY) (4), 585 (NY) (4); Breedlove 25021 (MO) (4), 25024 (MO) (4), 29980a (MO) (4); Britton 379 (NY) (4), 1142 (BM, DUKE, H, H-BR, NY) (5), 3982 (NY) (4), s.n. (BM) (5); Buck 5303 (NY) (5), 5563 (NY) (4), 5644 (NY) (4), 7604 (H, NY) (4), 7610 (NY) (4), 7909 (NY) (4), 8674 (NY) (5).

Callejas & Roldán 10618 (NY) (5); Callejas et al. 10110 (MO, NY) (5); Carlson 2486 (F) (4); Cazalet & Pennington 52 (NY) (5); Chaney s.n. (US) (4); Chávez 73 (MO) (4); Churchill & Betancur 18128 (NY) (5), 18150 (MO, NY) (5), 18664 (NY) (5), 18667 (NY) (5), 18673 (NY) (5), 18689 (MO, NY) (5), 18741 (MO, NY) (5); Churchill & Marulanda 15729 (NY) (5); Churchill & Rengifo 17496 (NY) (5), 17499 (NY) (5); Churchill & Sastre de Jesús 12903 (NY) (5), 12986-b (NY) (5), 12906 (DUKE, MO, NY) (5), 12990-b (MO, NY) (5), 12999 (NY) (5); Churchill et al. 14255 (H, MO, NY, US) (5), 14263 (NY) (5); Cleef et al. 302 (MO) (5); Clement 14535 (NY) (4); Clements 14 (NY) (4); Cook & Griggs 93 (NY, US) (4); Croat 41305 (MO) (4), 43391 (MO) (4); Crosby 2840 (MO) (4), 3203 (DUKE, MO) (4), 3415 (DUKE, MO) (5), 3429 (MO) (4), 3517 (DUKE, MO) (5), 3989 (MO) (4), 3704 (MO) (2), 9754 (MO) (3), 9761 (MO) (2), 9766 (MO) (2), 9789 (MO) (3), 9860 (MO) (5), 9928 (MO) (4), 9980 (MO) (4), 10109 (MO) (4), 10300 (MO) (4), 10861 (MO) (2), 10932 (MO) (2), 13128 (MO) (3), 13142 (MO) (2), 13771 (NY) (4), 13793 (NY) (4); Crosby & Crosby 5849 (DUKE, MO) (4), 5957 (MO) (2), 6005 (MO) (2), 6117 (DUKE, MO) (3), 6604 (MO) (3), 6608 (MO) (2), 8567 (MO) (4), 5836 (BM, DUKE, MO, NY) (2), 5860 (MO) (4), 6021 (MO) (4), 8523 (MO) (2); Crüger s.n. (NY, BM) (4); Crum 1068 (F, NY) (4).

Davidse & Zúniga 34760A (MO) (4); Delgadillo 1257 (MO) (4); Dorr & Barnett 6149 (NY) (5); Dorr et al. 5034 (NY) (5); Duncan s.n. (MO) (4); Duss 394 (NY) (4).

Elliot 7a (BM) (4), 8a (BM) (4), 22 (BM) (4), 69c (BM) (4), 98a (BM) (4), 1983 (BM) (4).

Germain 1147 (H-BR, NY) (5); Griffin et al. 92 (US) (3).

Hattaway et al. 112 (F, MO) (4); Herzog 4025 (BM, JE, MO, NY, PC) (5); Hioram 11940 (BM, F) (4), 12539 (F, MO) (4), 12699 (F) (4), 12757 (F) (4), 13620 (F) (4), 13697 (F) (4), 13756 (F) (4); Holm-Nielsen et al. 26952 (MO, NY) (5), 26962 (MO, NY) (5), 27145 (MO) (5), 27339 (MO, NY) (5); Holst 5831 (MO) (4), 5832 (MO) (4); Hooker 190 (NY) (4).

Johnson 794 (NY, US) (4); Juarez 981 (MO) (4), 1046 (MO) (4).

King C91–91 (MO) (4).

Laegaard 53628H (NY) (5); Laegaard & Luzano 18518 E (MO) (5); Leon 14559 (NY) (4); Leon & Clements 5476a (NY) (4); Lewis 83–710 (LPB) (5), 83–715 (LPB) (5); Liesner 26479 (MO, NY) (4), 26810 (MO) (4); Lyon 387 (MO) (4), 389 (MO) (4).

Marie 652 (NY) (4), 654 (NY) (4), 664 (BM) (4); Matuda s.n. (DUKE) (4); Maxon 9354 (MO, NY) (4); Maxon & Killip 160 (BM, F, NY, US) (4), 163 (BM, F, NY, US) (4); McPherson 13251D (MO) (5); Morton 7215a (US) (4); Morales 21429 (US) (3); Morales & Griffin 38 (MO) (3); Morton 5506 (US) (4), 7195 (US) (4), 7382 (US) (4), 7395 (US) (4), 9441 (US) (4); Morton & Acuna 3649 (US) (4); Murrill & Murrill 29 (NY) (4).

Nichols 37 (BM) (5), 179 (NY) (5), 184 (BM, NY) (5),

s.n. (DUKE) (5); Norris et al. 5613 (NY) (5), 5614 (NY) (5).

Olson 84–49a (MO) (4), 84–53 (MO) (4); Orcutt 7015 (US) (4).

Patterson & Bartram 19A (NY) (5); Price 834 (MO) (4), 839 (MO) (4); Pringle 10481 (BM, F, MO, NY, US) (4); Proctor 31252 (MO) (4).

Ramírez 2946 (NY) (5), 3056 (MO) (5), 10118 (MO) (5), 10205 (MO) (5), 10932 (MO) (5); Ramírez et al. 9039 (MO) (5), 10381 (MO) (5), 10405 (MO) (5); Ramírez & Salas 10863 (MO) (5); Reese 15745 (NY) (5); Richards & Sharp 7020 (F) (4); Robbins 89 (US) (5).

Salazar 694 (NY) (4); Salle s.n. (NY) (4); Schiffner 679 (BM) (1), 1122 (BM, H-BR) (1), 1454 (BM, H-BR) (1); Sharp 918 (US) (4), 2750 (US) (4), 3184 (US) (4), 3473 (US) (4), 3792-a (MO) (5), 3798 (F) (4), 3812-a (DUKE, F) (4), 5372 (DUKE, MO) (4), 5558 (US) (4), 5966 (US) (4), M59153 (US) (4), s.n. (US) (4), s.n. (US) (4); Sharp et al. 99-a (F) (4); 1863 (F) (4), 1872 (F) (4), 2831 (MO) (4), 3090 (F) (4), 4079 (F) (4), 4138 (F) (4), 4197 (F, MO) (4), 4207a (F) (4), 4209 (DUKE, F, MO, US) (4), 4367-f (F) (4), 4375 (MO) (1), 4507-b (MO) (4), 4520 (F, MO) (4); Shaw 5103 (NY) (4), 5765 (NY) (5); Smith et al. 2839 (F, MO) (4), 3079 (F) (4), 3117 (MO) (4); Spruce 1048 (BM, NY, H-BR) (5); Standley 4805 (BM, F, MO) (4), 4893 (F) (4), 4940 (F, MO) (4), 4954 (F) (4), 14112 (F) (4), 15659 (F) (4), 20676 (F) (4), 33697 (NY, US) (4), 42934b (US) (4), 47824 (NY, US) (4), 51185 (US) (4), 51185c (US) (4), 52598 (US) (4), 52898 (F) (4), 54633 (BM, F, NY, US) (4), 54644 (F, US) (4), 61818 (MO, F) (4), 64444 (MO) (4), 67444 (F) (4), 67450 (MO) (5), 67458 (F) (5), 67878 (F) (4), 70410a (F) (4), 80525 (F) (4), 85021 (F, NY, US) (4), 85022 (F) (4), 85024 (F) (4), 85577 (F) (4), 85926 (DUKE, F) (5), 85983 (F) (4), 85994 (F, NY) (5), 86406 (F, NY) (4), 91963 (F) (4); Standley & Torres 47592 (US) (4), 47653 (US) (4), 47877 (US) (4); Standley & Williams 707 (F) (4), 708a (F) (4); Steere 7668a (NY) (4), 7671 (MO, NY) (4), 9133 (NY) (5), 23073 (NY) (5), J-28 (NY) (4), J-86 (NY) (4); Stehle 7226 (US) (4); Stevens & Grijalva 15263 (MO) (4), 15265 (MO) (4); Steyermark 20056 (MO) (4), 31009 (DUKE, F, MO) (4), 33645 (F) (4), 33646 (F) (4), 34090 (F, NY) (5), 34324 (F) (4), 41919 (F) (4), 42557 (F) (4), 42656 (F) (4), 42660 (F) (4), 42661 (F) (4), 43226 (F, NY) (4), 46711 (F) (4), 48866 (F) (4), s.n. (NY) (4); Steyermark et al. 100692 (US) (5).

Thomas & Contreras 3765 (NY) (4); Thomas et al. 3571 (NY) (4); Türckheim 7374 (NY) (4), 7478 (BM) (4), 7507 (DUKE, NY) (4), s.n. (H-BR) (4).

Underwood 3377 (BM, NY, US) (4); Underwood & Earle 911 (NY, US) (4), s.n. (F) (4).

Watson ES-0022 (MO) (4); Wendland s.n. (BM, NY) (5), s.n. (BM) (5); Whittemore 1889A (MO) (5); Wright 112 (BM, H-BR, NY) (4), 113 (BM, NY) (4), 170 (NY) (4), 173 (DUKE, F, MO, US) (4).

Zanoni & Pimentel 20893T (MO, NY) (5); Zanoni et al. 25065-B (NY) (4).

RADIATION OF POLLINATION SYSTEMS IN *GLADIOLUS* (IRIDACEAE: CROCOIDEAE) IN SOUTHERN AFRICA[1]

Peter Goldblatt,[2] John C. Manning,[3] and Peter Bernhardt[4]

ABSTRACT.

Pollination strategies adopted by the largely sub-Saharan African *Gladiolus* (approximately 260 species), one of the largest genera of the monocot family Iridaceae, are unusually diverse. The primary or sole pollinators include long-tongued apid and anthophorine bees (Apidae), short-tongued halictid and andrenid bees (Halictidae, Andrenidae), sometimes in combination with hopliine beetles (Scarabaeidae), long-proboscid flies (Nemestrinidae, Tabanidae), large butterflies (Satyridae), moths (mostly Noctuidae and Sphingidae), and sunbirds (Passerinae). Floral form correlates closely with pollination strategy, allowing us to infer the pollination ecology of almost all 165 species in southern Africa, although we have observations of animal visitors capable of accomplishing pollen transfer in only half this number. Pollination by apid bees foraging for nectar and passively transferring pollen brushed onto their bodies during feeding occurs in all seven sections of the genus in southern Africa and is also the most common strategy in five of these sections. Other pollinators include female bees actively foraging for pollen, or long-proboscid flies, sunbirds, moths, the satyrid butterfly, *Aeropetes*, all foraging for nectar, and in one species hopliine beetles that use the flowers as sites for assembly and copulation. Shifts in floral form associated with changes in pollination appear complex in the large flowers of *Gladiolus* species, but may in fact involve relatively simple developmental modifications, involving changes in perianth pigmentation, and often the type of marking on the tepals, presence or absence of scent, length of the perianth tube, and occasionally a shift from zygomorphy to actinomorphy. Associated with these changes is a correlated adjustment in nectar characteristics, including volume, sugar concentration, and sometimes sugar chemistry. With an inferred minimum of 32 shifts in pollination system in the 165 species in southern Africa, *Gladiolus* appears to have an unusually labile floral morphology, which may account for its extensive adaptive radiation.

Key words: bees, butterflies, floral ecology, *Gladiolus*, Iridaceae, long-proboscid flies, moths, nectar, pollination systems, sunbirds.

The pollination ecology of most genera of African Iridaceae is remarkably diverse (Bernhardt & Goldblatt, 2000). For example, in *Sparaxis* four different pollination systems have been described in 13 of its 15 species studied (Goldblatt et al., 2000a). In general, the larger the genus the greater the diversity of pollination systems. Thus, in *Lapeirousia* (Goldblatt et al., 1995), with almost half the species in the genus studied, five different pollination systems have been identified among 20 species. For the genus *Ixia*, with an estimated 50 species, six different systems have been described for 20 species (Goldblatt et al., 2000b). The genus *Gladiolus*, with some 255 species in Africa, is thus a primary target for the analysis of pollination systems as it is the largest genus in the family in Africa (Goldblatt, 1996; Goldblatt & Manning, 1998).

Seven distinct and, with few exceptions, non-overlapping pollination systems (Table 1) occur among the 80 *Gladiolus* species studied to date, which suggests that the range of pollination systems in *Gladiolus* may be wider than in any other genus of the Iridaceae in Africa and probably in the entire family. A general review comparing the pollination systems in *Gladiolus* is useful for two reasons. First, as usual, significant literature is scattered throughout technical journals, popular publications, and scientific monographs. Second, because widely different pollination systems occur in closely related species, a review of pollination in *Gladiolus* may make a useful model system for expanding our understanding of the adaptations associated with pollination shifts and how often pollination mechanisms change within a plant lineage.

Gladiolus is believed to be monophyletic, as circumscribed by Goldblatt and Manning (1998). It is defined largely on non-floral characters, the flowers being highly variable as they reflect direct adap-

[1] Support for this study by grants 5408-95 and 5994-97 from the National Geographic Society is gratefully acknowledged. We thank B.-E. van Wyk, Rand Afrikaans University, Johannesburg, for the nectar chemistry data cited here.

[2] B. A. Krukoff Curator of African Botany, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, U.S.A.

[3] Compton Herbarium, National Botanical Institute, P. Bag X7, Claremont 7735, South Africa.

[4] Department of Biology, St. Louis University, St. Louis, Missouri 63103, U.S.A.

Table 1. Major floral types in *Gladiolus*. In pollen flowers tube length represents only the hollow (unblocked) upper part of the tube. Nectar sugar concentrations are ranges of means of samples of five to ten flowers in any species. Floral data are primarily from Goldblatt and Manning (1998); nectar data are from Goldblatt et al. (1998a, 1999) and Goldblatt and Manning (1999, 2002).

Flower type	Perianth shape	Perianth color	Perianth tube (mm)	Scent	Nectar volume (μl)	Nectar sugar conc. (%)	Dominant nectar sugar
Nectariferous bee	gullet or flag	Various, often bluish or pink, with dark streaks or yellow lip	12–20	Violet to rose or freesia	0.6–3.5(–6.0)	25–33(–40)	Sucrose
Pollen flower	rotate	Cream, yellowish or purple, without nectar guides	2.5–7	strong violet or none	ca. 0.5 or none	15.8	Sucrose
Long-proboscid fly	elongate gullet	Pink to cream with linear pink to red marks	(22–)30–100	none	2–12	25–30	Sucrose
Bird flower (*G. cunonius* clade)	gullet	Scarlet to crimson with green	12–20	none	6–27	18–27	Hexose
Bird flower (other clades)	flag (or gullet)	Scarlet to crimson	35–55	none	8–36	18–37	Sucrose
Moth flower	flag or gullet	White, cream or mottled brown	20–110	Sweet clove or fruity	4–12	(20–)30–36	Sucrose
Butterfly	flag (or gullet)	Scarlet to crimson, often with white splashes	35–55	none	4–20	18–27	Sucrose (rarely hexose)
Hopliine beetle	flag	Pink to orange with bold markings	ca. 10	Sweet violet	0.8–1.4	29.4	Sucrose

tations to particular pollination systems. The genus is defined by its unique ancestral basic chromosome number, $x = 15$, an inflated capsule, seeds with a broad circumferential wing, and a derived style in which the slender branches have expanded, bilobed tips that are conduplicate in bud and during the initial male phase of anthesis. The style appears to be the only floral feature that is apomorphic for the genus.

Distinctions between species, even those with different pollination systems, are relatively fine, although they appear highly visible in large-flowered plants such as *Gladiolus*. For example, the length and shape of the perianth tube, sometimes combined with a change in overall pigmentation, and details of the contrasting markings on the lower tepals (nectar guides) may signal a shift from apidanthophorine bee to bird or long-proboscid fly pollination (Goldblatt & Manning, 1998; Goldblatt et al., 1998a, 1999). Tube length may be as short as 2.5 mm to as long as 120 mm. Floral pigmentation and patterning are extraordinarily diverse and flowers may be virtually any color and bear diverse types of nectar guides, including longitudinal or transverse banding, diffuse speckling, or no apparent contrasting marks. Nectars are mostly sucrose-dominant, but a few species of *Gladiolus* sect. *Hebea* have hexose-dominant nectar. Most species have a tube with nectar in the lower half, but a few have the tube tightly enveloping the style and do not secrete nectar. Flowers may be zygomorphic with unilateral, arcuate stamens and style, or the perianth may be actinomorphic with the stamens and style either symmetrically arranged or unilateral.

Several of these differences appear so gross that the affinities of highly specialized species were, in the past, often misunderstood, and *Gladiolus* species were segregated in several different genera based on a range of floral features now known to be adaptations for particular pollination systems. Thus, *Acidanthera* included some *Gladiolus* species with white to pink, long-tubed flowers, and *Anomalesia, Homoglossum, Kentrosiphon,* and *Oenostachys* included species with bright red, long-tubed flowers, with the bracts, tepals, and floral tube modified in different ways. It is now known that *Acidanthera* was a polyphyletic assemblage of plants with flowers adapted for pollination by moths or long-proboscid flies. Its constituent species have been transferred to several genera, including *Babiana, Geissorhiza,* and *Hesperantha* (Goldblatt, 1984, 1985), although the type species is now included in *Gladiolus* (Goldblatt, 1996). In contrast, *Anomalesia, Homoglossum, Kentrosiphon,* and *Oenostachys* comprise species with flowers adapted for pollination by sunbirds (Goldblatt, 1996; Goldblatt & Manning, 1998), all now believed to be nested within different sections of *Gladiolus*. The reduction of these genera and the resulting reclassification of their species results in an entirely different picture of the adaptive radiation of a monophyletic group. What emerges is that in certain genera, of which *Gladiolus* is the prime example, the "correct" classification, that is, a monophyletic one, is vital to the interpretation of the adaptive radiation of a clade. In *Gladiolus* relatively minor structural modifications to suites of floral features cause pollination systems to change dramatically. In contrast to genera like *Pedicularis* (Scrophulariaceae), where changes in floral features alter the mode of pollination but not the pollinators (Macior, 1982, 1984), floral changes in *Gladiolus* often alter the pollen vectors radically, shifting pollination from one order of insects or birds to another.

Field studies of selected species of *Gladiolus* from different sections of the genus and including examples of several species of each of the main flower types show that flower type from whatever section closely correlates with pollination strategies (Johnson & Bond, 1994; Goldblatt et al., 1998a, b, 1999; Goldblatt & Manning, 1998, 1999, 2002). This allows us to infer pollination strategies of all but a few of the 165 species of *Gladiolus* that occur in southern Africa, site of most of the pollination studies so far conducted in the genus. Comparing pollination strategies in the genus with the phylogenetic classification proposed by Goldblatt and Manning (1998) makes it possible to infer, to a large extent, the patterns of floral radiation and associated pollination shifts that have occurred in the genus. This in turn permits us to gauge with some measure of confidence the evolutionary lability in pollination strategies and the extent to which ancestral floral morphology determines pollination system.

Floral Diversity in *Gladiolus*

Although species of *Gladiolus* exhibit a wide range of floral form and are particularly variable in perianth pigmentation, all share one important feature relating to their pollination ecology, a perianth tube. In most species, the tube forms a reservoir for nectar secreted from septal nectaries. Additionally, all but two of the southern African species (*G. quadrangulus* and *G. stellatus*) have unilateral stamens and styles, with the anthers normally exserted from the tube and arched below the dorsal tepal. Based on extensive field study and research for sys-

tematic monographs of the genus in tropical and southern Africa (Goldblatt, 1996; Goldblatt & Manning, 1998), we recognize seven major floral types, each with a different set of floral traits closely correlating with a particular pollination system. The most significant floral features include the shape and dimensions of the floral tube, perianth pigmentation, and the shape and color of the markings (nectar guides) on the lower tepals. Similar floral types do not always indicate shared relationship but are consistently associated with a particular pollination system (e.g., Goldblatt et al., 1995, 1999; Goldblatt & Manning, 1999; Manning & Goldblatt 1996, 1997). Vegetative morphology appears to be a more reliable guide to species relationships. The major floral types (Table 1) are as follows:

Group 1. Nectariferous bee flowers (Fig. 1A, B) include species with a zygomorphic, bilabiate perianth with an obliquely funnel-shaped tube mostly 12–20 mm long, usually slightly shorter than the arching to hooded dorsal tepal (gullet flowers sensu Faegri & van der Pijl, 1979) or sometimes the dorsal tepal is erect and prominent (flag flowers sensu Faegri & van der Pijl). The perianth tube consists of a flared upper portion that tapers to a narrow cylindrical lower half. Small amounts of nectar, secreted from septal nectaries, are retained in the lower, cylindrical half of the tube, which is usually 6–12 mm long. Nectar is sucrose-rich, ranges in volume from 0.6 to 3.5(to 6) μl per flower, and has a concentration mostly of 25–33%, but up to 40%, sucrose equivalents (88 species) (Goldblatt et al., 1998a).

Species of the southern African summer- and winter-rainfall zone show several differences among the flowers of this group. In the summer-rainfall zone, flowers are usually relatively small, ca. 20–25 mm long, rarely scented, and are numerous and crowded on straight spikes. The tepals are mostly colored shades of pink, orange, or mauve, and usually bear nectar guides of low contrast with the base perianth color (species of sects. *Densiflorus, Heterocolon, Linearifolius*, and *Ophiolyza*).

In the winter-rainfall zone flowers are medium-sized to relatively small and 25–35 mm long, are almost always scented, and are usually few to several, mostly on flexuose spikes. The tepals are often colored shades of blue to mauve or pink, but also scarlet, yellow, green, or brown, and the lower tepals usually bear prominent nectar guides of a variety of shapes, depending on taxonomic affiliation. Nectar guides may consist of uniform pale color on the proximal half of the tepals (sect. *Hebea*) or of pale color with irregularly streaked dark longitudinal lines and dots, or transverse bands of pale and dark color (sect. *Homoglossum*, sect. *Linearifolius*).

Group 2. Pollen flowers (Fig. 1C, D) are characteristically small, ca. 20 mm long, with an actinomorphic, mostly rotate perianth, and a tube less than 8 mm long or if longer completely closed internally due to the walls of the tube being closely wrapped around the style. The tepals are often dull-colored (cream, yellowish, or light purple), lack obvious nectar guides, and the stamens and style are unilateral or symmetrically placed in the center of the perianth. The flowers are scented or not, and secrete small to minute amounts of nectar or are nectarless. Species are restricted to winter-rainfall southern Africa in sections *Hebea, Homoglossum*, and *Linearifolius* and flower in the spring (4 species) (Goldblatt et al., 1998a).

Group 3. Long-proboscid fly flowers (Fig. 1E, F) have a medium to large perianth, mostly 45–80 mm long and an elongate, cylindrical perianth tube (22–)30–100 mm long, slightly to much exceeding the dorsal tepal. Few to several flowers are borne on straight spikes and have cream to pink tepals with reddish nectar guides of linear or spear-shaped marks in the middle of the tepals. The anthers and pollen are often unusually colored, and dark purple. Flowers always lack scent, but produce large amounts of sucrose-rich to sucrose-dominant nectar, mostly of 2–12 μl in volume and 25–30% sucrose equivalents per flower. Species occur throughout southern Africa, mostly of section *Blandus*, but there are also examples from sections *Densiflorus, Hebea, Homoglossum*, and *Ophiolyza* (29 species). Those of the winter-rainfall zone flower in late spring to summer, after peak flowering of the flora; in the summer-rainfall zone species flower in the late summer and autumn, coinciding with the end of the flowering peak in the flora (Goldblatt & Manning, 1999, 2000).

Group 4. Bird flowers (Fig. 1G, H) are represented by species with a large perianth, mostly 50–80 mm long, with an elongate tube, usually 35–55 mm long, as long as or longer than the dorsal tepal, the tube often narrow below, broad and cylindrical above (Table 1). The gullet or flag flowers lack nectar guides, are unscented, and the tepals are scarlet to crimson, sometimes partly yellow or green on the lower tepals. Plants often have well-exserted anthers borne on sturdy filaments, a stout stem, and an erect, straight spike bearing enlarged floral bracts. Flowers secrete large amounts of nectar, mostly sucrose-dominant (sects. *Ophiolyza* and *Homoglossum* series *Homoglossum*) and with 29–35% sucrose equivalents per flower, or sucrose rich (*G. priorii*: sect. *Homoglossum*) and 18–25% su-

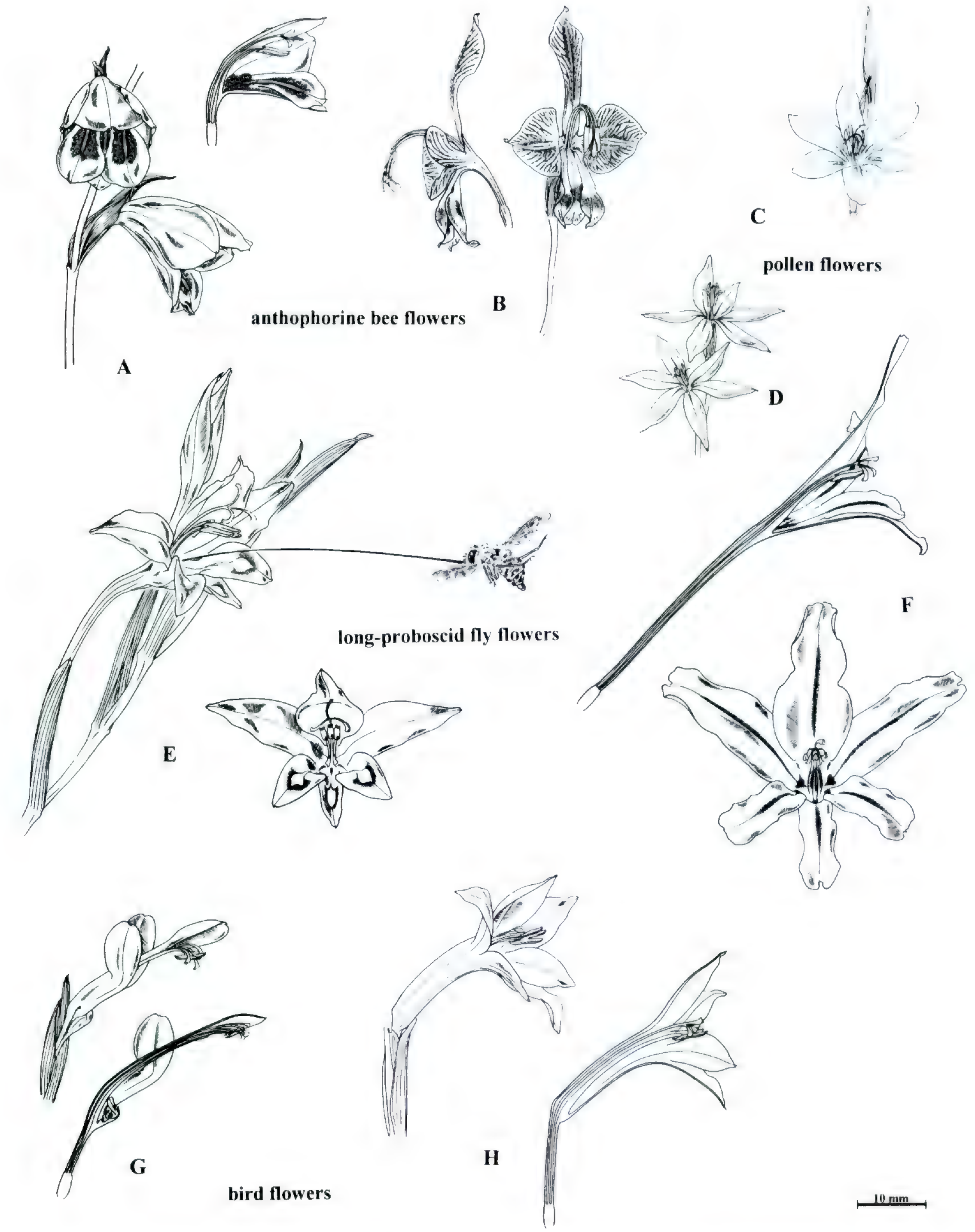


Figure 1. Examples of anthophorine bee flowers (A, B), pollen flowers (C, D), long-proboscid fly flowers (E, F), and bird flowers (G, H) in *Gladiolus*. —A. *G. papilio* (sect. *Densiflorus*). —B. *G. uysiae* (sect. *Hebea*). —C. *G. quadrangulus* (sect. *Homoglossum*). —D. *G. stellatus* (sect. *Hebea*). —E. *G. angustus* (sect. *Blandus*) and its pollinator, *Moegistorhynchus longirostris*. —F. *G. floribundus* (sect. *Blandus*). —G. *G. cunonius* (sect. *Hebea*). —H. *G. watsonius* (sect. *Homoglossum*). Scale bar 10 mm. Drawn by John Manning.

crose equivalents. Nectar volumes may be as much as 36 µl in *G. watsonius* (sect. *Homoglossum*) (Goldblatt & Manning, 1998; Goldblatt et al., 1999).

Flowers differ extensively among clades in this group. In section *Hebea*, bird pollinated species allied to *G. cunonius* (Table 1) have a perianth tube 12–20 mm long, the lower tepals reduced to scale-like dimensions, the dorsal tepal is much enlarged, the anthers are tailed, and the style branches stigmatic only at the rounded tips. They also produce relatively dilute nectar of 18–27% sucrose equivalents that contains a predominance of glucose and fructose, a sharp contrast to bird flowers of other sections. Bird pollinated *Gladiolus* species occur throughout southern Africa, mainly section *Homoglossum* in winter-rainfall southern Africa and flowering in the winter or spring, or section *Ophiolyza* in eastern southern Africa and flowering in the late spring and summer (20 species).

Group 5. Moth flowers (Fig. 2C—F) are found in species with a medium-sized to fairly large perianth and a cylindrical or gradually flared perianth tube, 20–110 mm long, somewhat to much exceeding the dorsal tepal (Table 1). The perianth is colored white to cream or alternatively, heavily speckled dull brown, with nectar guides obscure or evidently lacking (UV reflectance was not tested). Flowers are richly scented, sometimes only in the evening, and produce 4–12 µl of sucrose-dominant nectar, mostly 30–36%, but only 20–22% in *Gladiolus emiliae* and 24–28% in *G. longicollis*. An odd feature of several of these species are relatively short stamens, the anthers often partly included in the floral tube. Species are from four sections, notably series *Tristis* of section *Homoglossum*, and mostly restricted to winter-rainfall southern Africa, flowering from early spring to early summer, thus coinciding with the peak flowering of the flora (9 species) (Goldblatt & Manning, 1998, 2002).

Group 6. Satyrid butterfly flowers (Fig. 2A, B) are found in species with a large perianth, mostly exceeding 50 mm long, with a more or less cylindrical perianth tube and unilateral stamens, the latter included in the tube in *Gladiolus nerineoides*. The tube is slightly longer than the tepals, 35–55 mm long, but slender (as opposed to wide in the upper half in bird flowers). The tepals are reddish (scarlet to crimson), usually with white guides on the lower tepals (contrasting with absence of marking in bird flowers). The flowers are unscented, and produce ample amounts of sucrose-rich to sucrose-dominant nectar of moderate concentration, 18–27% sucrose equivalents and 4–20 µl in volume per flower (Table 1). Some species, including *G. cruentus* and *G. saundersii*, stand out among insect pollinated *Gladiolus* in having hexose-rich nectar. Flowering in the summer from mid-December to April, species occur mostly in winter-rainfall southern Africa, mostly of section *Blandus* but also section *Linearifolius*, with a few in summer-rainfall southern Africa (and tropical Africa), belonging to section *Ophiolyza* (9 species) (Johnson & Bond, 1994; Goldblatt & Manning, 1998, 2002).

Group 7. Hopliine beetle flowers occur in species with a moderate-sized perianth with a short perianth tube, ca. 10 mm long (Table 1). The tepals are brightly colored (either deep pink or orange) mostly with bold markings in yellow and purple (painted bowl flowers of Bernhardt, 2000) and show less pronounced zygomorphy, compared to immediate relatives, thus tepals are less unequal in size. Flowers secrete small amounts of nectar, bloom in the spring, and are restricted to winter-rainfall southern Africa (1 species) (Goldblatt et al., 1998b; Goldblatt & Manning, 1998).

Phenology

Flowering phenology is an important consideration in understanding the patterns of diversification of pollination systems in southern African *Gladiolus*. The subcontinent has two dramatically contrasting climate regimes, a warm wet summer and dry, cold winter in central and eastern southern Africa and a cool wet winter and hot, dry summer in the southwest. Species of *Gladiolus* occur in both zones but nearly all are restricted to only one. In the summer-rainfall zone species flower in the late spring, summer, and autumn, whereas in the winter-rainfall zone species may flower at any season. Even in the summer-dry winter-rainfall zone there are locally mesic habitats that support *Gladiolus* species flowering in the summer. Other summer- or autumn-flowering species bloom before foliage leaves are produced, an adaptation in the winter-rainfall zone that has significant consequences for pollination there.

Pollinator Classes and their Distribution

Field observations of floral visitors have shown a consistent pattern throughout southern Africa in the correlation between flower type and pollinator class, although individual species in each pollination class may differ, especially in the summer- versus the winter-rainfall parts of the subcontinent. The following pollinator classes have been recognized based on field observation, capture (excepting birds), and identification of visiting insects (Johnson & Bond, 1994; Goldblatt et al., 1998a; Gold-

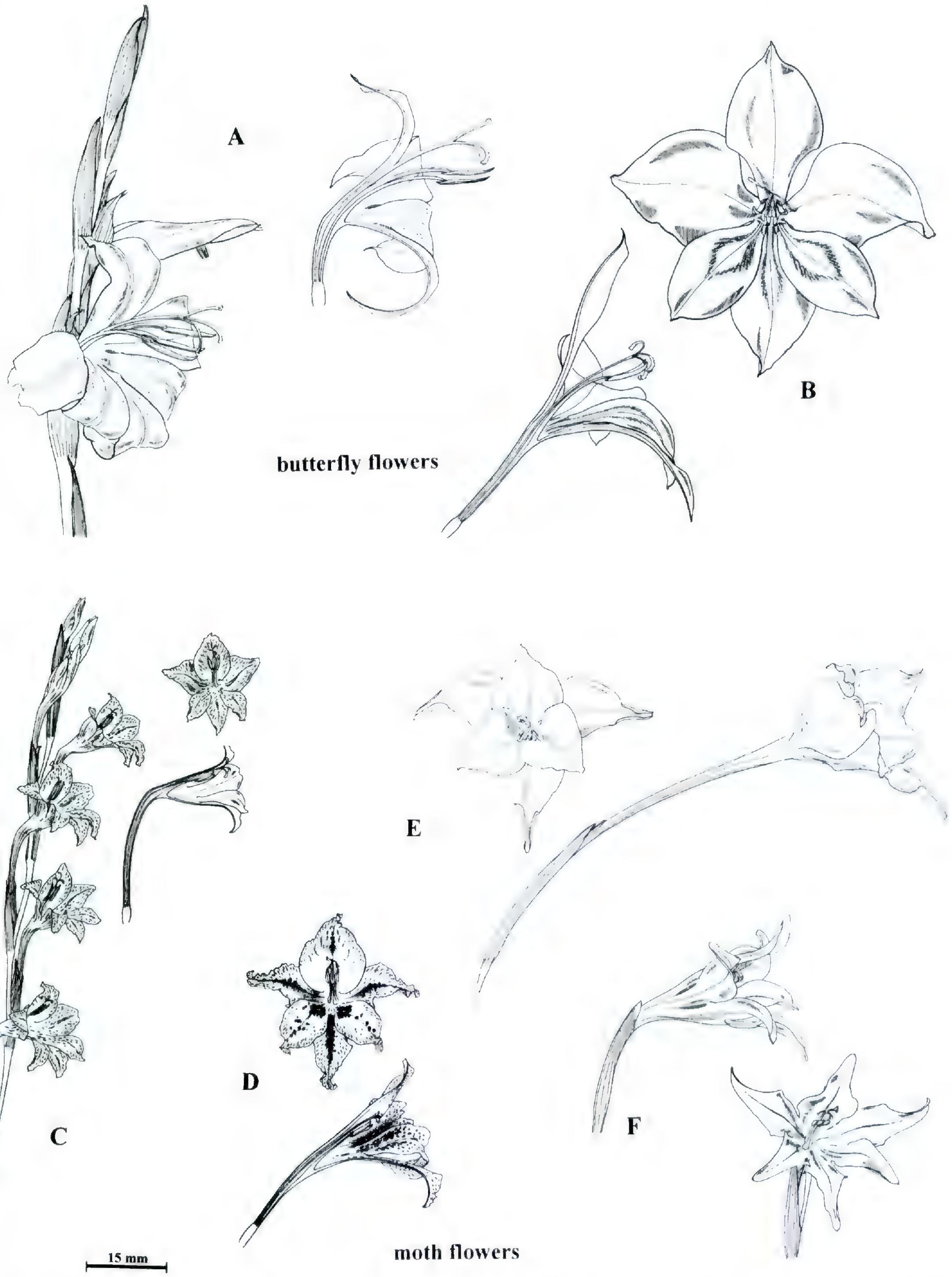


Figure 2. Examples of butterfly flowers (A, B) and moth flowers (C–F) in *Gladiolus*. —A. *G. saundersii* (sect. *Ophiolyza*). —B. *G. sempervirens* (sect. *Blandus*). —C. *G. emiliae* (sect. *Linearifolius*). —D. *G. maculatus* (sect. *Homoglossum*). —E. *G. longicollis* (sect. *Homoglossum*). —F. *G. tristis* (section *Homoglossum*). Scale bar 15 mm. Drawn by John Manning.

blatt & Manning, 1998, 1999; Manning & Goldblatt, 1995, and in prep.). Visitors were identified as pollinators (Table 2) when they had the appropriate size and shape to access floral nectar, brushed against the sexual organs of the flower during visits, and had been confirmed (by microscopic and sometimes direct visual examination) to be carrying pollen of the species visited on the appropriate part of their bodies so that stigmatic surfaces of flowers would brush against carried pollen on subsequent visits.

1. Long-tongued bees. Various large-bodied, long-tongued bees of the family Apidae s.l. (including Anthophoridae) (Roig Alsina & Michener, 1993), mainly *Amegilla*, *Anthophora*, sometimes *Apis*, and occasionally *Pachymelus*, *Tetralonia*, and *Xylocopa*, are the primary or exclusive visitors to 42 species (and are inferred for another 46 species) belonging to group 1, i.e., gullet or flag flowers of small to moderate size with tubes fairly short and obliquely funnel-shaped (Table 2). This is true irrespective of taxonomic grouping and phytogeography (Goldblatt et al., 1998a). These bees have bodies 10–17 mm long and 4–7 mm wide across the thorax, and mouthparts 4–10 mm long. Flowers show a close fit between size and shape and a bee's body. The flared upper tube is mostly 8–10 mm long and snugly accommodates a bee's head and thorax, while the narrow, cylindrical part, mostly 6–10 mm long, admits only the slender mouthparts. To reach the nectar, bees climb into the flower and push their mouth parts into the narrow part of the tube. As they maximally extend their tongues so that they can reach the nectar, their upper body is pressed against the anthers, which lie under the dorsal tepal. Pollen is then brushed onto the dorsal part of the thorax and sometimes the head or dorsal part of the abdomen. When stigmas are unfolded (then assumed to be receptive), usually on the last day a flower is open and when pollen has usually been removed (Goldblatt et al., 1998a), the stigmatic surfaces lie in the same position as the anthers, and are then ideally positioned for pollen to be deposited passively on their sticky surfaces as a bee visits the flower to feed on nectar.

Neither the base color of the flower nor the form of the contrastingly colored nectar guides appear significant in this pollination system, but the presence of nectar guides is so consistent that we assume it serves an important function, perhaps of orienting the bee so that it approaches the flower in a consistent manner so that pollen is effectively deposited dorsally on the body. The reward in these bee pollinated flowers is nectar, always present in moderate quantities and always sucrose-rich to sucrose dominant (Goldblatt et al., 1998a).

Often, the same bee species has been collected on flowers of a wide range of color and scent characteristics, suggesting that variation in size, pigmentation, scent, and form of the nectar guide outlined for group 1 has little direct significance in relation to pollinator. For example, the most common bee that we have recorded visiting *Gladiolus* flowers in western southern Africa in spring, *Anthophora diversipes*, visits species of almost any color and nectar guide configuration. Thus visual signals of ground color, nectar guide, and odor are not significant to pollinators. Likewise, the most common bees in eastern southern Africa, *Amegilla fallax* and *A. capensis*, both visit and can evidently pollinate many of the species there with flowers of this type.

In addition to bees, the flowers of group 1 are occasionally visited by the short-proboscid flies, *Psilodera* (Acroceridae) and *Prosoeca* (Nemestrinidae), with probosces 10–14 mm long. Their body and mouthpart size is similar to that of a large bee, and these nectar feeders function as pollinators just as effectively as long-tongued bees (Goldblatt et al., 1997, 1998a). Other visitors to these flowers include hopliine beetles (Scarabaeidae: Hopliini), which appear to be unimportant for most *Gladiolus* species, and we regard them as vandals rather than even secondary pollinators.

Except for a few isolated examples, notably *Gladiolus trichonemifolius* in which pollen appears to be an important reward, species are used mainly as sources of nectar and are visited by both male and female bees. Female bees use many other plants as pollen sources for nest provisioning (including co-blooming Asphodelaceae, Boraginaceae, Hyacinthaceae, Fabaceae, Malvaceae, Oxalidaceae, and Polygalaceae). The brighter and more varied coloration and frequent production of scent in the winter-rainfall zone versus the dull, less variable coloration and absence of scent in summer-rainfall eastern southern Africa (and tropical Africa), irrespective of taxonomic affiliation, is notable. This pattern holds even in section *Linearifolius*, the only section widely shared between the two areas. We have hypothesized that when flowers are dull-colored and appear (to the human eye) to blend with the surrounding vegetation and soil surface (Goldblatt et al., 1998a) scent may be the primary attractant. Otherwise, bright coloration combined with a distinctive odor may help flowers compete for pollinators in the winter-rainfall zone where flowering in the flora is concentrated in a few weeks in spring. In the summer-rainfall zone the flower

season is more protracted, and fewer species bloom at any particular time.

The significance of floral odor is often underestimated. Bees learn to discriminate among different odors six times more rapidly than they do colors (Schoonhoven et al., 1998) and can readily distinguish between numerous, similar odors. Thus the presence and variety of scents among co-blooming species alone may encourage floral constancy even when flowers are visually similar.

This type of bee pollination is the most common pollination strategy in *Gladiolus* (Table 3). It occurs in all seven taxonomic sections, and it is the most common one in sections *Densiflorus*, *Hebea*, *Heterocolon*, *Homoglossum*, and *Linearifolius*. Some 53% of the southern African species have flowers adapted for this system (Tables 3, 4). Elsewhere, we have postulated that anthophorine bee pollination is ancestral in *Gladiolus* (Goldblatt et al., 1998a; Goldblatt & Manning, 1999).

2. Short-tongued bees or worker honey bees. The short-tongued bee, *Andrena* sp., visits the fragrant flowers of *Gladiolus stellatus*, while *Apis mellifera* is the only bee species captured on actinomorphic *G. quadrangulus* and zygomorphic *G. aureus*. Both bees actively collect pollen, prominently displayed in erect anthers. In *G. stellatus* the flowers are particularly strongly scented. Flowers of *G. brevitubus* are zygomorphic, with unilateral stamens, but have a floral tube less than 3 mm long and evidently lack nectar. Only small halictid bees, ca. 6 mm long, have been captured on these flowers, which appear to offer pollen as the sole reward to insect visitors. Although short-tongued andrenid bees have been captured while visiting *Gladiolus meliusculus*, hopliine beetle species are more consistent and frequent visitors and so, we assume, are more important in the pollination of this species (Goldblatt et al., 1998b). This is discussed in detail below.

Pollination by small- or large-bodied female bees foraging for pollen as the primary pollination strategy is evidently found in only four southern African species of the genus (Goldblatt et al., 1998a). The system occurs in section *Hebea* (*Gladiolus stellatus*), section *Homoglossum* (*G. quadrangulus*: series *Carinatus*, and *G. brevitubus*: series *Brevitubus*), and section *Linearifolius* (*G. aureus*), evidently having evolved convergently four times (Table 4).

3. Long-proboscid flies. Long-proboscid flies with mouthparts mostly 20–60 mm long in two families, Nemestrinidae (tangle-veined flies) and Tabanidae (horseflies), have proven to be important pollinators (Table 2) of many southern African plant species with long floral tubes (Goldblatt et al., 1995; Manning & Goldblatt, 1996, 1997; Goldblatt & Manning, 1999). Long-proboscid flies have been found to be the sole visitors to *Gladiolus* species belonging to group 3, i.e., odorless, cream to pink flowers with an elongate tube and producing ample, sucrose-rich to -dominant nectar (Table 1). Typically only one species of fly at a particular site, occasionally two, visits a species. In the eastern southern African highlands, the late summer-flying *Prosoeca ganglbaueri* is the sole pollinator of at least *G. oppositiflorus*, *G. microcarpus*, *G. mortonius*, and *G. varius*. A fifth species, *G. calcaratus*, with a somewhat shorter tube, is also visited by this fly, but its tongue is so long that its body does not brush the anthers when it forages for nectar. The long-proboscid fly *Prosoeca robusta*, also on the wing in late summer, appears to be the main pollinator of *G. calcaratus*. Another fly, *Stenobasipteron wiedmannii* (also Nemestrinidae), is the sole visitor and presumably pollinator of the long-tubed *G. macneilii* in lower-altitude habitats in eastern southern Africa where *P. ganglbaueri* and *P. robusta* seem absent.

Along the south coast of South Africa the nemestrinid fly *Prosoeca longipennis* appears to be the sole pollinator of the long-tubed *Gladiolus bilineatus* and *G. engysiphon* that flower in the autumn. In western southern Africa, species with flowers of group 3 are visited by the tabanid *Philoliche rostrata* (*G. floribundus*, *G. monticola*, *G. undulatus*, *G. vigilans*), the nemestrinids *Moegistorhynchus longirostris* (*G. angustus*) and *M.* sp. (*G. rhodanthus*), either *Prosoeca nitidula* and *Philoliche rostrata* or both (*G. carneus*, *G. monticola*). Remarkable among long-proboscid flies, *M. longirostris* has a proboscis up to 80 mm long and the *Gladiolus* species that it pollinates, *G. angustus*, has a cream perianth with red markings and a tube 80–100 mm long.

Long-tongued flies are present in southern Africa mostly in the warmer months, October to April, and hence plant species pollinated by these insects are constrained to this flowering period. Furthermore, different species of fly are active at different times of the year. These flies are nectar feeders, and they acquire pollen loads from the flowers they visit passively. The remarkable similarity in flower color and the shape of the nectar guides in unrelated species of *Gladiolus* pollinated by long-proboscid flies suggests that there is an important adaptive value to floral conformity in long-tongued fly pollination systems.

Sixteen species of *Gladiolus* have to date been confirmed as being pollinated by long-proboscid flies (Table 3). An additional 13 species with similar floral morphology are inferred to have the same pollination strategy, thus 29 species, 18% of south-

Table 2. *Gladiolus* species and their confirmed pollinators and other floral visitors. Abbreviations: Coleop = Coleoptera, Dipt = Diptera, Hym = Hymenoptera, Lepid = Lepidoptera, Nemestr = Nemestrinidae, Passer = Passerinae, Scarab = Scarabaeidae. Data are from Goldblatt et al. (1998a, 1999) and Goldblatt & Manning (1999, 2000) except for the pollinator of *G. debilis*, *Prosoeca westermannii*, which is a novel report herein.

Species	Primary pollinator	Taxonomic affinity (order and family)	Secondary pollinator(s)	Thieves & vandals
Section *Densiflorus*				
G. appendiculatus G. J. Lewis	*Amegilla aspergina*	Hym: Apidae	—	*Prosoeca robusta*
G. calcaratus G. J. Lewis	*Prosoeca robusta*	Dipt: Nemestr	*Amegilla capensis*	*Prosoeca ganglbaurii*
G. crassifolius Baker	*Amegilla capensis*,	Hym: Apidae	*Prosoeca* sp.	—
	A. spilostoma	Hym: Apidae		
G. densiflorus Baker	*Amegilla fallax*	Hym: Apidae	—	*Prosoeca robusta*
G. exiguus G. J. Lewis	*Amegilla fallax*,	Hym: Apidae	—	*Prosoeca robusta*
	A. spilostoma	Hym: Apidae		
G. ferrugineus Goldblatt & J. C. Manning	*Amegilla aspergina*,	Hym: Apidae	—	—
	A. capensis	Hym: Apidae		
G. macneilii Oberm.	*Stenobasipteron wiedmannii*	Dipt: Nemestr	—	—
G. microcarpus G. J. Lewis	*Prosoeca ganglbaurii*	Dipt. Nemestr	—	—
G. mortonius Herbert	*Prosoeca ganglbaurii*	Dipt: Nemestr	—	—
G. papilio Baker	*Amegilla aspergina*,	Hym: Apidae	—	—
	A. capensis,	Hym: Apidae		
	Tetralonia sp.	Hym: Apidae		
G. varius F. Bolus	*Prosoeca ganglbaurii*	Dipt: Nemestr	*P. robusta*	—
Section *Ophiolyza*				
G. dalenii van Geel	*Nectarinia afra*,	Aves: Passer	—	—
	N. famosa	Aves: Passer		
G. dolomiticus Oberm.	*Amegilla spilostoma*,	Hym: Apidae	*Lasioglossum* sp.	*Allodape variegata*
	Xylocopa rufitarsus	Hym: Apidae		
G. ecklonii Lehm.	*Amegilla aspergina*,	Hym: Apidae	—	—
	A. capensis	Hym: Apidae		
G. flanaganii Baker	*Nectarinia famosa*	Aves: Passer	—	—
G. oppositiflorus Herbert	*Prosoeca ganglbaurii*	Dipt: Nemestr	—	—
G. saundersii Hook. f.	*Aeropetes tulbaghia*	Lepid: Satyridae	—	—
G. sericeovillosus Hook. f.	*Amegilla spilostoma*	Dipt: Nemestr	*Prosoeca* sp.	—
Section *Blandus*				
G. angustus L.	*Moegisorhynchus longirostris*	Dipt: Nemestr	—	—
G. bilineatus G. J. Lewis	*Prosoeca longipennis*	Dipt: Nemestr	—	—
G. cardinalis Curt.	*Aeropetes tulbaghia*	Lepid: Satyridae	—	—

Table 2. Continued.

Species	Primary pollinator	Taxonomic affinity (order and family)	Secondary pollinator(s)	Thieves & vandals
G. carneus D. Delaroche	*Philoliche rostrata*,	Dipt: Tabanid	—	*Amegilla spilostoma*
	Prosoeca nitidula	Dipt: Nemestr		
G. floribundus Jacq.	*Philoliche rostrata*	Dipt: Tabanid	—	—
	and/or *P. gulosa*	Dipt: Tabanid		
G. grandiflorus Andrews	*Anthophora diversipes*	Hym: Apidae	—	—
G. phoenix Goldblatt & J. C. Manning	*Amegilla spilostoma*	Hym: Apidae	—	—
G. sempervirens G. J. Lewis	*Aeropetes tulbaghia*	Lepid: Satyridae	—	—
G. stefaniae Oberm.	*Aeropetes tulbaghia*	Lepid: Satyridae	—	—
G. undulatus L.	*Philoliche rostrata*	Dipt: Tabanid	—	—
Section *Heterocolon*				
G. marlothii G. J. Lewis	*Anthophora diversipes*	Hym: Apidae	—	—
G. mostertiae L. Bolus	*Amegilla obscuriceps*	Hym: Apidae	—	—
G. rufomarginatus G. J. Lewis	*Amegilla langii*,	Hym: Apidae	—	—
	A. spilostoma,	Hym: Apidae		
	Stenobasipteron difficile	Dipt: Nemestr		
Section *Linearifolius*				
G. aureus Baker	*Apis mellifera*	Hym: Apidae	—	—
G. brevifolius	*Amegilla obscuriceps*,	Hym: Apidae	*Allodape exoloma*	*Lipotriche* sp.
	A. spilostoma,	Hym: Apidae	*A. pictifrons*	
	Psilodera valida	Dipt: Acrocerid		
G. emiliae L. Bolus	*Hippotion celerio*,	Lepid: Sphingid	—	—
	Cucullia extricata	Lepid: Noctuid		
G. guthriei F. Bolus	*Cucullia inaequalis*	Lepid: Noctuid	—	*Macaria simplicilinea*, *Hypagoptera* sp.
G. hirsutus Jacq.	*Apis mellifera*	Hym: Apidae	—	*Anisonyx ursus*
G. monticola G. J. Lewis ex Goldblatt & J. C Manning	*Philoliche rostrata*,	Dipt: Tabanid	—	*Amegilla spilostoma*
	Prosoeca nitidula	Dipt: Nemestr		
G. nerineoides G. J. Lewis	*Aeropetes tulbaghia*	Lepid: Satyridae	—	—
G. rhodanthus J. C. Manning & Goldblatt	*Moegistrohynchus* sp.	Dipt: Nemestr	—	—
Section *Hebea*				
G. alatus L.	*Anthophora diversipes*	Hym: Apidae	*Rediviva aurata*	—
G. arcuatus Klatt	*Apis mellifera*	Hym: Apidae	—	—

Table 2. Continued.

Species	Primary pollinator	Taxonomic affinity (order and family)	Secondary pollinator(s)	Thieves & vandals
G. ceresianus L. Bolus	*Anthophora diversipes*	Hym: Apidae	—	—
G. cunonius (L.) Gaertn.	*Nectarinia chalybea*	Aves: Passer	—	—
G. equitans Thunb.	*Pachymelus peringueyi*	Hym: Apidae	—	—
G. meliusculus (G. J. Lewis) Goldblatt & J. C. Manning	*Lepisia rupicola.*	Coleo: Scarab	*Andrena* sp.	—
	Pachycnema crassipes	Coleo: Scarab		
G. orchidiflorus Andrews	*Anthophora diversipes*	Hym: Apidae	—	—
G. permeabilis D. Delaroche	*Anthophora diversipes*	Hym: Apidae	—	—
G. saccatus (Klatt) Goldblatt & M. P. de Vos	*Nectarinia famosa,*	Aves: Passer	—	—
	N. fusca	Aves: Passer		
G. scullyi Baker	*Anthophora diversipes*	Hym: Apidae	—	—
G. speciosus Thunb.	*Rediviva aurata*	Hym: Melittidae	—	—
G. stellatus G. J. Lewis	*Andrena* sp.	Hym: Andrenidae	—	—
G. uysiae L. Bolus ex G. J. Lewis	*Anthophora diversipes*	Hym: Apidae	—	—
G. venustus G. J. Lewis	*Anthophora diversipes,*	Hym: Apidae	—	—
	A. krugeri	Hym: Apidae		
G. virescens Thunb.	*Anthophora diversipes*	Hym: Apidae	—	—
G. watermeyeri L. Bolus	*Anthophora diversipes*	Hym: Apidae	—	—
	A. krugeri	Hym: Apidae		
Section *Homoglossum*				
G. abbreviatus Andrews	*Nectarinia famosa*	Aves: Passer	—	—
G. blommesteinii L. Bolus	*Anthophora diversipes*	Hym: Apidae	—	—
G. brevitubus G. J. Lewis	*Lasioglossum* sp.	Hym: Halictidae	—	—
G. carinatus Aiton	*Anthophora diversipes*	Hym: Apidae	*Lasioglossum* sp.	—
	A. krugeri,	Hym: Apidae		
	A. schulzei	Hym: Apidae		
	Apis mellifera	Hym: Apidae		
G. debilis Sims	*Prosoeca westermannii*	Dipt: Nemestr	—	—
G. engysiphon G. J. Lewis	*Prosoeca longipennis*	Dipt: Nemestr	—	—
G exilis G. J. Lewis	*Amegilla fallax*	Hym: Apidae	—	—
G. gracilis Jacq.	*Anthophora diversipes,*	Hym: Apidae	*Xylocopa rufitarsus*	*Anisonyx ursus*
	Apis mellifera	Hym: Apidae		
G. griseus Goldblatt & J. C. Manning	*Apis mellifera*	Hym: Apidae	—	—
G. inflatus Thunb.	*Amegilla obscuriceps*	Hym: Apidae	—	—
G. inflexus Goldblatt & J. C. Manning	*Anthophora krugeri,*	Hym: Apidae	—	—

Table 2. Continued.

Species	Primary pollinator	Taxonomic affinity (order and family)	Secondary pollinator(s)	Thieves & vandals
	Apis mellifera,	Hym: Apidae		
	Patellapis sp.	Hym: Halictidae		
G. jonquilliodorus Eckl. ex G. J. Lewis	*Amegilla spilostoma*,	Hym: Apidae	—	—
	Apis mellifera	Hym: Apidae		
G. liliaceus Houtt.	*Cucullia extricata*	Lepid: Noctuidae	—	*Tyromoptes inferior*
G. longicollis Baker	*Agrius convolvuli*	Lepid: Sphingidae	—	—
G. maculatus Sweet	*Cucullia terensis*	Lepid: Noctuidae	—	—
G. martleyi L. Bolus	*Amegilla spilostoma*	Hym: Apidae	—	—
G. meridionalis G. J. Lewis	*Nectarinia violacea*	Passer: Nectarinid	—	—
G. quadrangulus (D. Delaroche) Barnard	*Apis mellifera*	Hym: Apidae	—	—
G. patersoniae F. Bolus	*Anthophora diversipes*,	Hym: Apidae	*Apis mellifera*	—
	A. krugeri	Hym: Apidae		
G. recurvus L.	*Hippotion eson*	Lepid: Sphingidae	*Anthophora diversipes*	—
G. sufflavus (G. J. Lewis) Goldblatt & J. C. Manning	*Anthophora diversipes*	Hym: Apidae	—	—
G. trichonemifolius Ker-Gawl.	*Apis mellifera*	Hym: Apidae	—	—
G. tristis L.	*Cucullia* sp.	Lepid: Noctuidae	—	—
G. vigilans Barnard	*Philoliche rostrata*	Dipt: Tabanidae	—	—
G. virgatus Goldblatt & J. C. Manning	*Philoliche rostrata*	Dipt: Tabanidae	—	—

Table 3. Analysis of the frequency and taxonomic distribution of the pollination systems in southern African species of *Gladiolus*. Southern African species comprise 165 species distributed in 7 sections and 27 series, all believed to be monophyletic based on morphological analysis (Goldblatt & Manning, 1998); n/a = not applicable.

Pollination System	Total species confirmed	Total confirmed and inferred	Taxonomic sections	Taxonomic series
Large-bodied, long-tongued bees (Apidae s.l.)	39	87 (53%)	7	22
Short-tongued (or pollen-collecting) bees	4	4 (2%)	3	3
Long-proboscid fly	16	29 (18%)	6	13
Passerine bird	6	20 (12%)	4	7
Moth	6	11 (7%)	3	5
Satyrid butterfly	5	9(5%)	3	3
Hopliine beetle (short-tongued bee)	1	1 (<1%)	1	1
Uncertain	4	4 (2%)	n/a	n/a

ern African *Gladiolus*, are inferred to be adapted for pollination by long-proboscid flies. Long-proboscid fly pollination thus appears to be the second most common pollination strategy in the genus, after nectariferous bee pollination (Goldblatt & Manning, 1999, 2000). The strategy occurs in six of the seven sections of the genus and it is the most common one in section *Blandus*, elsewhere occurring in isolated species. In section *Densiflorus* long-proboscid fly pollination occurs in one or two species of three series, in sections *Homoglossum* and *Hebea* in three species each belonging to a different series, and in one species of sections *Linearifolius* and *Ophiolyza*. For the present, long-proboscid fly pollination is assumed to have arisen only once in any series, though this is by no means established. This suggests parallel evolution of long-proboscid fly pollination a minimum of 12 times.

4. Sunbirds. *Gladiolus* flowers adapted for pollination by sunbirds have been identified in five sections of the genus (Goldblatt et al., 1999). The flowers conform to the classic type in Africa that are pollinated by sunbirds, *Nectarinia* (Table 2), a genus of passerine birds that feed on nectar as well as insects (Rebelo et al., 1987). Although the flowers in species of each section are broadly similar in their red color and production of large amounts of nectar, they differ in significant details, including perianth tube shape, size of the lower tepals relative to the dorsal, presence or absence of contrasting markings, and nectar sugar chemistry (Goldblatt & Manning, 1998).

In section *Homoglossum* all six species of series *Homoglossum* and two of series *Mutabilis* appear to have flowers adapted for sunbird pollination. These species all have a long tube, the upper part of which is wide and cylindrical (presumably to accommodate a bird's bill), and the species produce fairly concentrated nectar that is sucrose-dominant, a stark contrast to the bird flowers in section *Hebea* and many other bird pollinated flowers. Most of the remaining species of *Gladiolus* in southern Africa

Table 4. Taxonomic distribution of pollination systems in southern African *Gladiolus*. Lp fly = Long-proboscid fly.

Gladiolus section (total species)	Pollination system—known/plus predicted							
	Bees							
	Passive	Active	Lp fly	Bird	Moth	*Aeropetes*	Hopliine beetle	Uncertain
Section *Densiflorus* (20)	6/12	0	5/8	0	0	0	0	0
Section *Ophiolyza* (15)	2/6	0	1/1	2/5	0	1/2	0	1
Section *Blandus* (21)	2/6	0	5/8	0/1	0	3/5	0	1
Section *Linearifolius* (17)	2/7	1/1	2/2	0/2	2/2	1/2	0	1
Section *Heterocolon* (9)	3/8	0	0	0	0	0	0	1
Section *Hebea* (32)	12/21	1/1	0/3	2/4	0/2	0	1/1	0
Section *Homoglossum* (51)	12/27	2/2	3/7	2/8	4/7	0	0	0
Total 165	39/87	4/4	16/29	6/20	6/11	5/9	1/1	4
% total	53%	2%	18%	12%	7%	5%	<1%	2%

adapted for bird pollination belong in section *Ophiolyza*, the most well known of which is the widespread *G. dalenii* (also in Madagascar and tropical Africa). This species has been reported by Vogel (1954) to be visited by *Nectarinia afra* (greater double-collared sunbird), and we recorded *N. famosa* foraging actively on *G. dalenii*. The high Drakensberg *G. flanaganii* has also been recorded as visited by *N. famosa*. Observations of visits by birds to the six species of series *Homoglossum* are limited to sightings of *N. famosa* visiting *G. abbreviatus*. In section *Hebea*, visits to *G. saccatus* (a western southern African species) by *Nectarinia famosa* and *N. fusca* and to *G. cunonius* (a southern coastal species) by *N. chalybea* have been noted (Goldblatt & Manning, 1998). We did not try to capture avian visitors of any bird pollinated species but confined ourselves to observation of visitors and analysis of floral nectar.

Bird pollinated flowers occur in five species of section *Ophiolyza*, four species in one series of section *Hebea*, eight species in two series of section *Homoglossum*, two species in one series of section *Linearifolius*, and one species of section *Blandus*, a total of 20 species, 12% of the southern African species. In series *Mutabilis* of section *Homoglossum* it is not yet established whether the bird pollinated species *G. meridionalis* and *G. priorii* are a clade or are independently derived from a common ancestor (Goldblatt & Manning, 1998). In bird pollinated species, markedly different inflorescence or floral adaptations confirm the independent origin of the strategy. In section *Hebea* three species of bird pollinated flowers have the style branches apically stigmatic and anthers with long sterile tails, in addition to hexose-rich to hexose-dominant nectar, unique adaptations in the genus. In section *Homoglossum* series *Homoglossum* the spike is straight and relatively thick, whereas in series *Mutabilis* the spike is fairly slender and flexuose (an ancestral feature found in other members of this section). For want of firm evidence to the contrary, we assume that bird pollinated members of section *Ophiolyza* are a monophyletic group and represent a single origin of the strategy. We hypothesize that bird pollination arose at least six times in *Gladiolus* and possibly seven.

5. Night-flying moths. A variety of moths have been captured visiting species with flowers of group 5, and included species of two families, Noctuidae and Sphingidae (Table 2). The syndrome is difficult to document because the moths are active at night and hence difficult to catch or even to see. Sphinx moth pollination occurs in *G. longicollis* in eastern southern Africa (*Agrius convolvuli*), both sphinx and noctuid moths (species of the genera *Hippotion* and *Cucullia*) pollinate several species of the winter-rainfall west of the subcontinent, and species of the noctuid *Cucullia* have been captured on *G. guthriei*, *G. liliaceus*, and *G. maculatus*. An unidentified species of Sphingidae also visited *G. liliaceus* but avoided capture. We saw few other insects visiting any putatively moth pollinated species during many hours of observation, day and night, but a male anthophorid bee, *Anthophora diversipes*, was captured while visiting *G. recurvus* (I. Nänni, pers. comm.), a species apparently adapted for moth pollination. The bee attempted to forage on nectar of this species, flowers of which are scented during the day as well as the night. The bee is evidently not the normal visitor but appears capable of accomplishing pollination although unable to reach the nectar in the long perianth tube.

Moths are poor pollen vectors, as their bodies are covered with loose scales to which pollen appears to adhere loosely. However, all the moths captured carried visible amounts of *Gladiolus* pollen on the upper parts of their probosces (Goldblatt & Manning, 2002). When the flowers are in the female phase, on the last of four or five days of anthesis, the stigmatic lobes lie in the same position as the anthers, and pollen adhering to a moth's proboscis is then optimally placed for passive transfer to the exposed stigma lobes. There seems no obvious distinction between species pollinated by sphingids versus noctuids except perianth tube length. In the longer-tubed *G. longicollis*, tube length, 65–110 mm long in subspecies *platypetalus*, prevents successful nectar foraging by most noctuids.

Some 11 southern African species in three different sections of *Gladiolus* (7% of the southern African total—Tables 3, 4) have flowers of group 5, and observations on six of them (Goldblatt & Manning, 1998, and in prep.) confirm that they can correctly be categorized as being adapted for moth pollination. Moth pollinated flowers are inferred for two species of section *Hebea* (*G. robertsoniae* and *G. acuminatus*, which according to morphological comparison (Goldblatt & Manning, 1998) are distantly related members of series *Permeabilis*); section *Homoglossum* (*G. maculatus*, *G. albens*, and morphologically very different and presumably distantly related *G. recurvus*: series *Gracilis*, and four of the five species of series *Tristis*); and section *Linearifolius* (*G. emiliae* and *G. guthriei*: series *Linearifolius*). We suggest that the strategy most likely arose six times, once in section *Linearifolius*, twice in section *Hebea* and three times in section *Homoglossum*.

6. Large butterflies. A single species of butterfly, *Aeropetes tulbaghia* (Satyridae), appears to pollinate species of group 6, those with red flowers, usually with white markings on the lower tepals. This butterfly has an innate affinity for bright red colors and is the sole or major pollinator of a guild of red-flowered species that bloom in the late summer in southern Africa (Johnson & Bond, 1994). We have recorded *Aeropetes* visiting *G. cardinalis*, *G. nerineoides*, *G. saundersii*, *G. sempervirens*, and *G. stefaniae*. Both *G. cardinalis* and *G. sempervirens* have already been reported in the literature to be pollinated by *Aeropetes* (Johnson & Bond, 1994). Demonstration of this pollination syndrome is difficult because population sizes of *Aeropetes* vary considerably from year to year, and individuals may be absent locally in some seasons. Hence, populations of some species of *Gladiolus* may not be visited and pollinated at all in some years. We assume this to be the case in 1995 when we attempted to identify the pollinator of *G. cruentus* and *G. insolens*, which we infer from floral morphology to be adapted for pollination by *Aeropetes* (or in the case of *G. cruentus*, perhaps some other large butterfly).

Butterfly flowers in *Gladiolus* superficially resemble those adapted for bird pollination. Butterflies and birds do not, however, normally share any *Gladiolus* species, and in the winter-rainfall part of southern Africa they cannot, for no bird pollinated *Gladiolus* species there flowers when *Aeropetes* is on the wing in late summer. Apart from phenology, flowers pollinated by *Aeropetes* have a narrower tube than do flowers pollinated by birds and so do not permit entry of a bird's bill. The white splashes on most *Gladiolus* species pollinated by *Aeropetes* are not a feature of any bird pollinated members of the Iridaceae excepting *G. flanaganii*, and this is the only species of the genus in which both *Aeropetes* and sunbirds are recorded as visitors (Johnson & Bond, 1994). The firm texture of the floral parts, rigid stem, and hooded dorsal tepal suggest sunbird pollination is its primary strategy. At least in the winter-rainfall zone butterfly flowers appear to have evolved from fly pollinated ancestors where the phylogenetic relationships can be inferred, as in series *Blandus* (Goldblatt & Manning, 1998).

As in long-tongued bee, long-proboscid fly, and moth pollination systems, the reward offered to butterflies is nectar on which these insects feed, and pollen transfer is passive. Sugar concentration is normally somewhat lower than in other insect pollination systems (Johnson & Bond, 1994; Goldblatt & Manning, 1998), mostly 18–24% sucrose equivalents, but volume is high and sugars are typically sucrose-rich to -dominant. The exceptions are *G. cruentus* and *G. saundersii*, which have hexose-rich to hexose-dominant nectar (Goldblatt & Manning, 2002).

Some 9 species, 5% of the southern African species, may be inferred as being adapted for *Aeropetes* pollination (Table 3). Species belong to three sections (*Blandus*: 5 spp., *Linearifolius*: 2 sp., and *Ophiolyza*: 2 spp.—Table 4). Thus, we hypothesize the origin of the strategy a minimum of three times. Except in series *Linearifolius*, floral morphology of *Aeropetes* pollinated flowers is remarkably similar and is associated with large flowers with spreading tepals and white splashes on the lower tepals. In section *Linearifolius*, *G. nerineoides* has relatively small flowers, but several flowers are usually open at the same time, providing the display comparable to one large flower found in sections *Ophiolyza* and *Blandus*.

7. Hopliine beetles. These beetles of the family Scarabaeidae use the flowers of a range of plant families for sites of assembly, mate selection, and copulation (Steiner, 1998; Goldblatt et al., 1998b) and sometimes consume pollen or other plant materials. Flowers most commonly favored by these beetles are salver- or bowl-shaped, actinomorphic, and although variously colored, typically have dark contrasting markings, sometimes called beetle marks (painted bowl flowers of Bernhardt, 2000). The only *Gladiolus* species that approaches this pattern is *G. meliusculus*, which has pink flowers with enlarged lower tepals that form a comparatively large platform. The lower tepals have a broad, dark transverse band and are yellow at the tips. The pigmentation broadly mimics that of two species of *Romulea* (Iridaceae) that often grow sympatrically and are visited by the same beetle species. However, *G. meliusculus* has a zygomorphic flower that produces a floral odor and a short perianth tube containing measurable amounts of nectar, and it is also visited by *Andrena* sp., a short-tongued bee. Too few visits by bees were observed for us to assess their importance in the pollination of this species. It is possible that the bee is at best an occasional visitor, unlike the beetles, which could consistently be found on flowers of these species at our study sites. The floral form of *G. meliusculus* suggests a recent shift to hopliine pollination, and it does not have the classic appearance associated with species pollinated by hopliines.

Discussion

Much of the evolutionary radiation in Iridaceae has involved changes in floral features, and it

comes as no surprise that floral morphology in the family is closely correlated with pollination strategy. These are often very precise and involve a narrow range of pollinators, often a single pollinator group or only one pollinator (Table 2). The seven taxonomic sections of *Gladiolus* (Table 4) recognized in southern Africa by Goldblatt and Manning are each considered to be monophyletic, as are their constituent series (Goldblatt & Manning, 1998), and they are defined by unique, derived characters.

NECTAR

Two surprising results have emerged from an analysis of nectars produced by *Gladiolus* flowers (Johnson & Bond, 1994; Goldblatt et al., 1998a, 1999; Goldblatt & Manning, 1999, unpublished data). Irrespective of taxonomic affinity and, with two exceptions, of pollinator, nectars are sucrose-rich to sucrose-dominant. The exceptions are a lineage of three bird pollinated species of section *Hebea*, *G. cunonius*, *G. saccatus*, and *G. splendens*, and two species of section *Ophiolyza* inferred to be butterfly pollinated, *G. cruentus* and *G. saundersii*, which have hexose-rich to hexose-dominant nectar. The development of hexose-rich nectar in section *Hebea* is consistent with the presumed taste preferences of passerine birds (Baker & Baker, 1990). However, comparable evolution of hexose-rich nectar is conspicuously absent in the four other sections of southern African *Gladiolus* that contain bird pollinated species. The development of hexose-rich nectar in section *Ophiolyza* is likewise noteworthy, as it is unusual for butterfly flowers in the Iridaceae (unpublished data), though not unique. *Hesperantha (Schizostylis) coccinea*, a presumed butterfly flower, has hexose-dominant nectar (Johnson & Bond, 1994) in contrast to the bee or moth pollinated members of that genus (Goldblatt et al., in press).

In general, nectar sugar chemistry in *Gladiolus* appears to reflect phylogenetic relationship rather than pollinator preference, as has been reported, for example, in African Asphodelaceae and Ericaceae (Percival, 1961; van Wyk et al., 1993; Barnes et al., 1995). However, natural selection by pollinators presumably accounts for the shift to hexose-rich nectar in section *Hebea* of *Gladiolus*. *Aeropetes* is, however, not known to have a preferred type of nectar, so the shift to hexose-rich nectar in butterfly pollinated species of section *Ophiolyza* is puzzling.

Nectar concentration seems to be loosely linked to pollinator (Kevan & Baker, 1983; Baker & Baker, 1990), and this is to a limited extent reflected in *Gladiolus*. Flowers of *Gladiolus* pollinated by apid-anthophorine bees or moths typically have relatively concentrated nectar, between 30% and 36% sucrose equivalents. Species pollinated by long-proboscid flies typically have slightly more dilute nectar, 20% to 30% (Goldblatt & Manning, 1999, 2000). Butterfly flowers have moderate sugar concentrations, in *Gladiolus* between 18% and 26%. The latter pattern is consistent with Johnson and Bond's (1994) observations on flowers pollinated by *Aeropetes*.

Flowers pollinated by nectarinids, however, show no apparent pattern in *Gladiolus*. Bird pollinated species of series *Homoglossum* have sugar concentrations ranging from 28% to 33%, but in series *Mutabilis* of the same section as low as 21–26% in *G. priorii* and 37% in *G. meridionalis*, the latter remarkable in bird pollinated flowers. In contrast, bird pollinated species of section *Ophiolyza* have nectar mostly of 18–20% concentration except for *G. flanaganii*, which has nectar of 35% concentration. This absence of pattern is likewise consistent with Johnson and Bond's (1994) observations on flowers pollinated by nectarinids. Avian nectar feeders tend to favor more dilute nectars (Kevan, 1984; Baker & Baker, 1990; Nicholson, 1998), and this is reflected in the nectar of many *Gladiolus* species. The high nectar concentration in the species of series *Homoglossum* and *G. meridionalis* may reflect heightened calorific requirements in sunbirds in the winter, when temperatures are low and breeding occurs. In wet winter conditions rain may also cause dilution of nectar (Nicholson, 1998), and the high nectar concentration in these *Gladiolus* species may, alternatively and simply, be an adaptation to counteract the dilution effect.

Nectar volume, as might be expected, is closely linked to pollinator size, itself linked to flower size (Baker & Baker, 1990). The larger the pollinator the larger the flower and the greater the amount of nectar. Thus bird flowers have the highest quantities of nectar, and bee (and hopliine) flowers the lowest. Butterfly and long-proboscid fly flowers fall between the extremes. Hopliine and small-bodied bee pollinated flowers either produce reduced amounts of nectar or none at all. *Gladiolus* flowers, then, appear to be excellent sources of nectar for the particular pollinator for which they are adapted and floral changes associated with shifts in pollination system are tracked by nectar volume and concentration. Nectar sugar chemistry seems more conservative and is largely sucrose-rich to -dominant as it is in most members of subfamily Crocoideae (syn. Ixioideae) (Goldblatt et al., 1995, 1999, 2000a, 2000b, in press; Manning & Goldblatt,

1996, 1997). The marked trend away from this pattern in one bird pollinated lineage of section *Hebea*, which has hexose-rich to hexose-dominant nectar, seems likely to be pollinator driven. This same pattern occurs in several bird pollinated lineages of the family, including *Klattia* and *Witsenia* (Nivenioideae) and *Chasmanthe* (Crocoideae), but not in others (Goldblatt, 1993; Johnson & Bond, 1994; Goldblatt et al., 1999).

POLLINATOR SHIFTS AND EVOLUTION IN THE GENUS

In their analysis of the radiation of pollination systems in *Disa* (Orchidaceae) in southern Africa, Johnson et al. (1998) asked two questions. One, did each system originate once or did each mode have several independent origins? Two, does history play an important role in determining the pollination biology of a species or are pollination systems evolutionarily labile? These questions can equally be asked of *Gladiolus*, and the answers are strikingly similar. As in *Disa*, there is ample evidence from comparative morphology and cladistic analysis that different pollination systems arose repeatedly within *Gladiolus*. Thus, we conclude that, within certain parameters, pollination systems in the genus are extremely labile. There is always a historical component to the radiation of pollination systems, and the zygomorphic, tubular, often large, nectar-producing flowers of *Gladiolus* seem constrained to utilizing pollination systems that involve passive pollen transfer with pollinators visiting flowers in search of nectar. This contrasts markedly with genera like *Hesperantha*, *Ixia*, and *Romulea*, which have small, rotate, or campanulate flowers that emphasize pollination systems involving hopliine beetles or bees foraging for pollen, or a combination of hopliines and bees. Thus, hopliine beetles, which favor actinomorphic, salverform flowers; butterflies, other than *Aeropetes*; bombyliid flies, which favor relatively small flowers; and carrion flies; all significant pollinators of the southern African flora, are not at all or are or barely utilized within *Gladiolus*. Even the active pollination system involving female bees foraging for pollen is weakly developed, although it has evolved independently in four species of four different sections or series (*G. aureus*, *G. brevitubus*, *G. quadrangulus*, and *G. stellatus*).

It has been implicitly assumed that explicit phylogenetic hypotheses based on well-supported cladistic analyses are necessary to determine patterns of floral radiation and associated diversity of pollination systems in flowering plants (Armbruster, 1992, 1993; Johnson et al., 1998). We lack such a phylogeny in *Gladiolus*, but the monographs of the genus for tropical and southern Africa offer phylogenetic classifications and preliminary cladograms based on morphological analysis (Goldblatt, 1996, Goldblatt & Manning, 1998). In southern Africa, Goldblatt and Manning recognize seven sections in the genus (Table 4), each supported by specialized features (synapomorphies). Within these sections a total of 21 species aggregates (informal taxonomic series) are also recognized, these likewise supported by derived characters. Thus, a phylogenetic framework exists, which offers a broad understanding of the major patterns of radiation in the genus. Pollination biology in *Gladiolus* is also moderately well understood. Pollination by anthophorine bees and native *Apis mellifera* foraging for nectar and passively transporting dorsal loads of pollen is the most common system, now documented in 43 species. The close similarity of floral morphology and nectar characteristics suggest that an additional 44 species share this same pollination system (Table 3).

Pollination systems in *Gladiolus*, in order of importance (Table 3) then, are large-bodied, long-tongued bees (53%), long-proboscid flies with proboscs over 20 mm long (18%), nectarinid birds (12%), night-flying moths (7%), the satyrid butterfly, *Aeropetes* (5%), and small-bodied, short-tongued female bees foraging for pollen (2%). The common hopliine beetle system of southern African Iridaceae is represented in just one species. Matching the pollination strategy against the classification of the genus shows repeated shifts in pollination system (Table 4). Assuming that large-bodied, long-tongued bee pollination is ancestral, as postulated above, we suggest a minimum estimate of the independent origin of long-proboscid fly pollination at least 12 times (in six sections of the genus). Likewise, we infer the independent origin of passerine pollination 7 times (in 7 series in four sections), moth pollination 5 times (in 5 series in 3 sections), and *Aeropetes* pollination 3 times (in 3 series in 3 sections). Combining this with the independent origin of active bee pollination in four species in four separate series and hopliine pollination in one series, we infer a total of at least 32 shifts in pollination system within *Gladiolus*. Put another way, this represents one shift for every 5 species of *Gladiolus*. This appears to be a remarkable degree of flexibility in floral characters and in patterns of convergent evolution, at least as far as current knowledge allows this statement.

PATTERNS OF SPECIATION—GEOGRAPHY VERSUS POLLINATION SYSTEM

A more difficult question to answer than the frequency of pollinator shifts is why these shifts oc-

curred. Two factors appear significant in pollinator shifts in *Gladiolus*. One frequent pattern is an associated shift in soil substrate without any significant geographic disjunction among closely related species with different pollinators. Several examples illustrate this point. In the four species of series *Floribundus*, bee pollinated *G. rudis* and *G. grandiflorus* grow on sandstone or clay slopes respectively, fly pollinated *G. floribundus* on drier rocky sandstone habitats, and bird pollinated *G. miniatus* is restricted to coastal limestones. In series *Permeabilis*, *G. permeabilis* subsp. *edulis* favors sandy or rocky doleritic-derived soils, whereas the immediately related *G. sekukuniensis* is restricted to dolomite and other limestone-type soils (Manning et al., 1999). In the three species of series *Appendiculatus*, bee pollinated *G. appendiculatus* occurs on igneous substrates, while the two fly pollinated species, *G. calcaratus* and *G. macneilii*, occur on sandstone- or dolomite-derived soils, respectively. A similar pattern is mirrored in series *Gracilis*, where moth pollinated *G. maculatus* favors clay soils, while the closely related *G. priorii* and *G. meridionalis*, both bird pollinated, favor rocky sandstone or granite slopes (*G. priorii*) or coastal limestones (*G. meridionalis*). As in *Lapeirousia* (Goldblatt & Manning, 1996), one pattern of species diversification in *Gladiolus* is marked by an edaphic shift accompanied by a change in pollinator without a marked geographic disjunction.

A second pattern of speciation is associated with a shift in flowering phenology. In the southern African winter-rainfall zone this shift is sometimes combined with a change in pollinator in closely related species. A phenological shift is consistently associated with *Aeropetes* pollination because this butterfly is on the wing from late December to April. At least three clades comprise species that flower in the late summer or autumn and show a shift to *Aeropetes* pollination. This phenological shift need not accompany a change in pollinator. Several autumn-flowering species of sections *Homoglossum* and *Linearifolius* maintain ancestral large-bodied anthophorine bee pollination but flower at a time when few other members of the surrounding flora are in bloom, and competition for bee pollination must be minimal. In the winter-rainfall zone, related species flower at the expected time, earlier in the season. In the summer-rainfall zone there is a comparable phenological shift in species of section *Linearifolius*, in this case for flowering earlier in the season, at the end of the dry season and before the flowering peak in the flora. Phenological shifts, whether accompanied by pollinator shifts or not, are generally accompanied by changes in patterns of vegetative growth (Goldblatt & Manning, 1998). Most often the production of leaves is delayed until conditions are favorable for vegetative growth, and leaves present at flowering time are reduced in size and often in number. Alternatively, the flowering stem with its reduced leaves does not die as the seeds mature, but remains green throughout the growing season.

Long-proboscid flies are on the wing late in the flowering season, mainly October to December in the winter-rainfall zone, February to April in the summer-rainfall zone. Shifts to this pollination system may be driven by selection by these insects. An aspect of pollination by extreme specialists, either long-proboscid flies or *Aeropetes*, that awaits explanation is the prominence of these systems as soon as these insects are on the wing. Bees are the predominant pollinators in the winter-rainfall zone early in the season (late winter and spring) and in the late spring and early summer in the summer-rainfall zone, but their importance for genera like *Gladiolus* falls dramatically once long-proboscid flies and then *Aeropetes* become active. It may be that competition for conventional pollinators (apid-anthophorine bees) influences the shift to specialist pollinators. There is, however, no evidence that bee populations also fall at this time, which might lead to the exploitation of alternative pollinators. It may simply be that long-proboscid flies, which usually carry pure loads of pollen of particular species at various sites on their bodies (Goldblatt & Manning, 1999, 2000) are more effective pollinators. Bees remain active and plentiful in the summer-rainfall zone thoughout the time that long-proboscid flies and *Aeropetes* are active. In the winter-rainfall zone apid bees also remain active and prominent pollinators of *Gladiolus* species that flower in late summer and autumn.

As noted for subgenus *Lapeirousia* in winter-rainfall southern Africa (Goldblatt & Manning, 1996), edaphic shifts appear to be the primary step in population differentiation, which is then reinforced by a pollinator shift. This may be more readily accomplished in small peripheral populations separated ecologically from the swamping effects of the ancestral gene pool. The second pattern, which involves a phenological shift, may be entirely pollinator driven. Whatever the full explanation may be, there is no doubt that pollinator shifts explain much of the species diversity in the African genera of the Iridaceae.

Species diversification within the same pollination system requires different explanations. Some species fit the classic pattern of divergence caused by geographic isolation alone. For example, within

series *Homoglossum* the lineage of similar-flowered, bird pollinated species, *G. quadrangularis*, *G. teretifolius*, and *G. watsonius*, occupy separate, although adjacent geographic ranges, but maintain identical soil preferences, flowering times, and pollinators (Goldblatt & Manning, 1998). A comparable pattern is evident in the second lineage of the series, in which *G. abbreviatus*, *G. fourcadei*, and *G. huttonii* have adjacent ranges across the southern and eastern Cape, but there is a shift in phenology in *G. abbreviatus* to winter-flowering as compared to its spring-flowering relatives.

Speciation patterns are often more complex. Competition for the same suite of pollinators at times of flowering peaks, August and September in the winter-rainfall zone and December to February in the summer-rainfall zone, is one explanation for the presence of species clusters in sections *Densiflorus*, *Hebea*, and *Homoglossum*, all pollinated by the same range of large-bodied bees. Under intense competition for pollinators, a distinctive display of form, color, or even fragrance may enhance reproductive success. In a genus such as *Gladiolus*, where species are self-incompatible but are almost all interfertile, only highly distinctive species sharing the same pollinator can coexist in a particular habitat, whatever their edaphic niche may be, without the development of hybrids. It is not unusual to find three or four coblooming species of *Gladiolus* sharing the same set of bee species as their pollinators. Each *Gladiolus* species has its own microhabitat, so that competition for space and nutrients is not a concern. However, in such situations species invariably differ substantially in appearance and fragrance. Anthophorine bees, which are to a degree flower constant (Bernhardt, 1996; Goldblatt et al., 1998b), do not visit these different species sequentially, and hybridization is rare in our experience, and unknown in undisturbed habitats. We therefore assume that introgression is not an explanation for much if any of the species diversity in the genus. Reproductive isolation due to polyploidy or dysploidy can also be largely ruled out as having played a role in the radiation in southern African *Gladiolus*. Nearly all species are diploid and have the same chromosome number, $n = 15$ (Goldblatt & Takei, 1997). Only *G. dalenii* is consistently polyploid in southern Africa (diploid races occur in tropical Africa and Madagascar), and *G. leptosiphon*, $n = 30$, may be a polyploid species, although only one population has been examined for chromosome number (Goldblatt et al., 1993; Goldblatt & Takei, 1997). The predominant factors that promote reproductive isolation in *Gladiolus* then are floral presentation frequently combined with edaphic or phenological shifts, phenological shifts alone, or conventional geographic barriers to dispersal.

SPECIALIST POLLINATION SYSTEMS AND RARITY

As pointed out by Johnson and Bond (1994) for species pollinated by *Aeropetes*, many of the plants are rare or have narrow ranges. This is certainly true of butterfly pollinated species of the winter-rainfall zone, but it is also true that these species are usually restricted to rare habitats that remain moist during the dry summer and autumn (Goldblatt & Manning, 1998). Long-proboscid fly pollinated species of the winter-rainfall zone also flower fairly late and require mesic habitats that limit their distribution. Thus the rarity or narrow ranges of species using specialist pollinators may be no more than a reflection of the scarcity of suitable habitats available to them compounded by the difficulty of dispersal to isolated habitats where they can become established. The correlation of specialist pollinator and rarity is misleading. It is probably not the result of the unusual pollination system but of the paucity of suitable habitats and the difficulty of dispersal to similar habitats, located considerable distances away.

In the southern African summer-rainfall zone some *Gladiolus* species are certainly rare. While the immediately related *G. permeabilis* is widespread across southern Africa, *G. sekukuniensis* is restricted to a narrow stretch of Northern Province. Similarly, *G. macneilii* is a narrow endemic, whereas allied and presumably ancestral *G. appendiculatus* has a far wider range. However, both these rare species are also edaphic specialists, confined to unusual limestone soils. The correlation here, then, is a reflection of edaphic shift being associated with a change in pollinator.

Conclusion

The ultimate explanation for the success of *Gladiolus* compared to related genera in the family seems to lie in a genetic system that allows for adaptation to many different habitats, soil types, and climatic conditions combined with an extremely labile floral form that is receptive to selection. If there is one feature of the reproductive system that appears distinctive in *Gladiolus* it is that most species appear, at least from preliminary studies (Goldblatt et al., 1998a; Goldblatt & Manning, 1999), to be self-incompatible, unlike other genera in the same subfamily, and moreover, the flowers are strongly protandrous and herkogamous, also unlike the situation in other Crocoideae (where this is

known). The genus also has a high basic chromosome number, $x = 15$, one of the highest in the family. Outcrossing and associated genic recombination are therefore maximized. The only other genus that is comparable in the family, *Moraea* (subfamily Iridoideae), is also an unusually large genus with some 200 species, and it too shows strong self-incompatibility, protandry and herkogamy, but not a high basic chromosome number. Whatever the explanations may be for the species richness in *Gladiolus*, and they are probably numerous and complex, we can only marvel at the diversity and adaptability of this remarkable genus.

Literature Cited

Armbruster, W. S. 1992. Phylogeny and the evolution of plant-animal interactions. BioScience 42: 12–20.

———. 1993. Evolution of plant pollination systems: Hypotheses and tests with the Neotropical vine *Dalechampia*. Evolution 47: 1480–1505.

Baker, H. G. & I. Baker. 1990. The predictive value of nectar chemistry to the recognition of pollinator types. Israel J. Bot. 39: 157–166.

Barnes, K., S. W. Nicholson & B.-E. van Wyk. 1995. Nectar sugar composition in *Erica*. Biochem. Syst. Ecol. 23: 419–423.

Bernhardt, P. 1996. Anther adaptation in animal pollination. Pp. 192–220 *in* W. G. D'Arcy & R. C. Keating (editors), The Anther: Form, Function, and Phylogeny. Cambridge Univ. Press, Cambridge, U.K.

———. 2000. Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. Pl. Syst. Evol. 222: 293–320.

——— & P. Goldblatt. 2000. The diversity of pollination mechanisms in the Iridaceae of southern Africa. Pp. 301–308 *in* K. Wilson & P. Weston (editors), Systematics and Biology of the Monocots. Royal Botanic Gardens, Sydney, Australia.

Faegri, K. & L. van der Pijl. 1979. The Principles of Pollination Ecology, Ed. 3. Pergamon Press, New York.

Goldblatt, P. 1984. A revision of *Hesperantha* (Iridaceae) in the winter rainfall area of southern Africa. J. S. African Bot. 50: 15–141.

———. 1985. Systematics of the southern African genus *Geissorhiza* (Iridaceae–Ixioideae). Ann. Missouri Bot. Gard. 72: 277–447.

———. 1993. The Woody Iridaceae: Systematics, Biology and Evolution of *Nivenia*, *Klattia* and *Witsenia*. Timber Press, Portland, Oregon.

———. 1996. *Gladiolus* in Tropical Africa. Timber Press, Portland, Oregon.

——— & J. C. Manning. 1996. Phylogeny and speciation in *Lapeirousia* subgenus *Lapeirousia* (Iridaceae subfamily Ixioideae). Ann. Missouri Bot. Gard. 83: 346–361.

——— & ———. 1998. *Gladiolus* in Southern Africa. Fernwood Press, Cape Town.

——— & ———. 1999. The long-proboscid fly pollination system in *Gladiolus* (Iridaceae). Ann. Missouri Bot. Gard. 86: 758–774.

——— & ———. 2000. The long-proboscid fly pollination system in southern Africa. Ann. Missouri Bot. Gard. 87: 146–170.

——— & ———. 2002. Evidence for moth and butterfly pollination in the genus *Gladiolus* (Iridaceae: Crocoideae). Ann. Missouri Bot. Gard. 89 (In press).

——— & M. Takei. 1997. Chromosome cytology of Iridaceae, base numbers, patterns of variation and modes of karyotype change. Ann. Missouri Bot. Gard. 84: 285–304.

———, ——— & Z. A. Razzaq. 1993. Chromosome cytology in tropical African *Gladiolus* (Iridaceae). Ann. Missouri Bot. Gard. 80: 461–470.

———, J. C. Manning & P. Bernhardt. 1995. Pollination in *Lapeirousia* subgenus *Lapeirousia* (Iridaceae: Ixioideae). Ann. Missouri Bot. Gard. 82: 517–534.

———, ——— & ———. 1997. Notes on the pollination of *Gladiolus brevifolius* (Iridaceae) by bees (Anthophoridae) and bee mimicking flies (*Psilodera*: Acroceridae). J. Kansas Entomol. Soc. 70: 297–304.

———, ——— & ———. 1998a. Adaptive radiation of bee pollinated *Gladiolus* species (Iridaceae) in southern Africa. Ann. Missouri Bot. Gard. 85: 492–517.

———, ——— & ———. 1998b. Pollination by monkey beetles (Scarabaeidae–Hopliini) in petaloid geophytes in southern Africa. Ann. Missouri Bot. Gard. 85: 215–230.

———, ——— & ———. 1999. Evidence of bird pollination in the Iridaceae of southern Africa. Adansonia ser. 3, 21: 25–40.

———, P. Bernhardt & J. C. Manning. 2000a. Adaptive radiation of pollination mechanisms in the African genus *Ixia* (Iridaceae: Crocoideae). Ann. Missouri Bot. Gard. 89: 564–577.

———, ——— & ———. 2000b. Adaptive radiation of pollination systems in *Sparaxis* Ker Gawler (Iridaceae: Ixioideae). Adansonia sér. 3, 22: 57–70.

———, I. Nänni, P. Bernhardt & J. C. Manning. In press. Floral biology of *Hesperantha* (Iridaceae: Crocoideae): How minor shifts in floral presentation change the pollination system. Ann. Missouri Bot. Gard.

Johnson, S. D. & W. J. Bond. 1994. Red flowers and butterfly pollination in the fynbos of South Africa. Pp. 137–148 *in* M. Arianoutsou & R. Grooves (editors), Plant Animal Interactions in Mediterranean-Type Ecosystems. Kluwer Academic Press, Dordrecht.

———, H. P. Linder & K. E. Steiner. 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). Amer. J. Bot. 85: 402–411.

Kevan, P. G. 1984. Pollination by animals and angiosperm biosystematics. Pp. 271–292 *in* W. E. Grant (editor), Plant Biosystematics. Academic Press, Toronto.

——— & H. G. Baker. 1983. Insects as flower visitors and pollinators. Ann. Rev. Entomol. 28: 407–453.

Macior, L. W. 1982. Plant community and pollinator dynamics in the evolution of pollination mechanisms. Pp. 29–45 *in* J. A. Armstrong et al. (editors), Pollination and Evolution. Royal Botanic Garden, Sydney.

———. 1984. Behavioral coadaptation of *Bombus* pollinators and *Pedicularis* flowers. Vème Symposium International sur la Pollinisation. Colloq. I.N.R.A. 21: 257–261.

Manning, J. C. & P. Goldblatt. 1995. Cupid comes in many guises: The not so humble fly and a pollination guild in the Overberg. Veld & Flora 81: 50–52.

——— & ———. 1996. The *Prosoeca peringueyi* (Diptera: Nemestrinidae) pollination syndrome in southern Africa: Long-tongued flies and their tubular flowers. Ann. Missouri Bot. Gard. 83: 67–86.

——— & ———. 1997. The *Moegistorhynchus longi-*

rostris (Diptera: Nemestrinidae) pollination guild: Long-tubed flowers and a specialized long-tongued fly pollination system in southern Africa. Pl. Syst. Evol. 206: 51–69.

———, ——— & P. D. J. Winter. 1999. Two new species of *Gladiolus* (Iridaceae: Ixioideae) from South Africa and notes on long-proboscid fly pollination in the genus. Bothalia 29: 217–223.

Nicholson, S. W. 1998. The importance of osmosis in nectar secretion and its consumption by insects. Amer. Zool. 38: 418–425.

Percival, M. S. 1961. Types of nectar in angiosperms. New Phytol. 60: 235–281.

Rebelo, A., G. Siegfried & E. G. H. Oliver. 1987. Pollination syndromes of *Erica* species in the south-western Cape. S. African J. Bot. 51: 270–280.

Roig Alsina, A. & C. D. Michener. 1993. Studies of the phylogeny and classification of long-tongued bees (Hymenoptera: Apoidea). Univ. Kansas Sci. Bull. 55: 123–162.

Schoonhoven, L. M., T. Jermy & J. J. A. van Loon. 1998. Insect–Plant Biology. Chapman Hall, London.

Steiner, K. S. 1998. Beetle pollination of peacock moraeas in South Africa. Pl. Syst. Evol. 209: 47–65.

Vogel, S. 1954. Blütenbiologische Typen als Elemente der Sippengliederung. Bot. Stud. 1: 1–338.

Van Wyk, B.-E., C. S. Whitehead, H. F. Glen, D. S. Hardy, E. J. van Jaarsveld & G. F. Smith. 1993. Nectar sugar composition in the subfamily Alooideae (Asphodelaceae). Biochem. Syst. Ecol. 21: 249–253.

ANNALS OF THE MISSOURI BOTANICAL GARDEN: CHECKLIST FOR AUTHORS

Scope

The *Annals* publishes original articles in systematic botany and related fields. Papers whose purpose is the establishment of new nomenclatural entities in vascular plants and bryophytes are not accepted. Rather, they should be submitted to *Novon* for consideration. (*Novon* instructions are available on the Web at www.mobot.org or from the managing editor.)

Conditions for Publication

Authors are urged to contact the managing editor prior to submitting manuscripts. Because the diskette/electronic file will be used by the printer for typesetting, it is important to adhere to the items listed in the format section of the checklist. Authors' electronic files are formatted by the editors and are sent to the printer in electronic form (not as camera-ready pages).

Manuscripts that have been reviewed before submission will be subject to the full normal review process initiated here. Manuscripts not properly prepared may be returned for revision prior to review. It is the author's responsibility to supply missing bibliographic information; the editor does not perform library research except by prior arrangement with authors not having access to crucial reference materials. Communication with the editor at all points of the publication process is encouraged.

Page Charges

Page costs are $80 per page, although charges may sometimes be reduced. Changes in proof made by authors will be billed non-negotiably to the author at $3.50 per line changed.

Correspondence

Amy McPherson, Managing Editor
MBG Press, P.O. Box 299, St. Louis, MO 63166–0299, U.S.A.
Street address: MBG Press, Monsanto Center, 4500 Shaw Blvd., St. Louis, MO 63110-2291
Tel: (314) 577-5112; Fax: (314) 577-9594
Email: annals@mobot.org
www.mobot.org

1. General

- ☐ Text is in English or Spanish on numbered pages.
- ☐ Manuscript is submitted on MS-DOS/WINDOWS 3½ in. diskette, as Microsoft Word® [preferable] file.
- ☐ Three copies of double-spaced printed manuscript, including abstract, legends, tables, specimen lists, Literature Cited, and footnotes, are enclosed. The printed manuscript is the **same** as the document on the diskette.
- ☐ Photocopies of the figures are included with each copy of the manuscript. Copies of half-tone figures (photographs) and plates must be of sufficiently good quality to show all the detail of the originals. Digital or dye-sublimation proofs of electronic graphic files are submitted. [Send originals or photostats of originals when manuscript is accepted for publication.]
- ☐ Authorities, abbreviated according to Brummit & Powell's *Authors of Plant Names*, are provided for species' names the first time they are mentioned in the text: OR they are provided in a table that includes names of species.
- ☐ Letter is enclosed containing the author's full mailing address, telephone number, fax number, and e-mail address.

2. Format of Text

- ☐ Right-hand margin is not justified, and words are not hyphenated there.
- ☐ One space is inserted after periods, colons, question marks, and all other punctuation marks.
- ☐ First sentences of paragraphs are indented with a tab.
- ☐ Two hyphens with no space between them are used to indicate ranges of numbers, for example, page numbers, 20–23; measurements, 5–7 mm; altitudes, 1500–2500 m.
- ☐ A single hyphen is used in hyphenated words, for example, 9-nerved, oval-triangular.
- ☐ Common Latin words or phrases are not italicized (e.g., et al., i.e., sensu, etc.).
- ☐ Only taxon names at the rank of genus and below are italicized.
- ☐ Correct accents, umlauts, and other diacritical marks have been included.
- ☐ All figures and tables are cited in the text and are numbered in the order in which they are to appear.

3. Style

- ☐ Recent issue of the *Annals* is used as a model.
- ☐ *Chicago Manual of Style*, latest edition, is used as a reference.

4. First Page

- ☐ Footnotes are typed as double-spaced paragraphs on the first page. The first footnote contains acknowledgments, including information on granting agencies, herbaria that loaned specimens, and the name of the artist. The second footnote is the author's address. Addresses for additional authors are each separate footnotes. No other footnotes occur, except in tables, where unavoidable.

5. Abstract & Key Words

- ☐ A one-paragraph abstract precedes text. Papers in Spanish have an English abstract in addition to a Spanish resumen.
- ☐ The abstract is concise (1 paragraph) and includes brief statements about the paper's intent, materials and methods, results, and significance of findings.
- ☐ A brief list of key words immediately follows the Abstract.

6. Taxonomic Treatment

- ☐ Species entries are organized as follows: Heading, Latin diagnosis (if necessary), description, distribution, vernacular name(s), discussion, specimens examined. The discussions are parallel and follow the same order, e.g., diagnostic characteristics, distinction from similar species, variation, distribution and ecology, nomenclature and typification, uses.
- ☐ One paragraph per basionym is used as follows: *Taxon* author, literature citation, type citation, e.g., **Beilschmiedia latifolia** (Nees) Sa. Nishida, Ann. Missouri Bot. Gard. 86: 680. 1999. *Hufelandia latifolia* Nees, Syst. Laur.: 674. 1836. TYPE: Peru. Locality not indicated, 1835 (fl), *Matthew 1433* (holotype, E!; isotypes, BM!, E!, K!, LE not seen, OXF not seen).
- ☐ Synonyms based on different types are placed in separate paragraphs, each beginning with the basionym, followed by other combinations (if appropriate), and citation of the type.
- ☐ A brief Latin diagnosis for each new taxon is provided rather than a complete Latin description.
- ☐ For species with infraspecific taxa: Description and discussion are composite (incorporating all infraspecific taxa) and parallel with other species descriptions. Descriptions of infraspecific taxa are parallel with one another (in the same species). All synonyms are listed under the appropriate infraspecific taxon.
- ☐ Descriptions: Descriptions are parallel, within a given rank. All measurements are metric. Hyphens are used for parenthetical extremes: "peduncle (8.2–)14.3–28.0(–31.9) cm long," unless intermediate values are not expected: ovary with (2)4(6) locules. Length × width are given in the following manner: lamina 36.4–82.8 × 9.1–16.8 cm.
- ☐ When relevant, nomina nuda, misapplied names, and superfluous names are included in the discussion following the description, but are not part of the formal synonymy.

7. Citation of Types

- ☐ Exclamation points are used for specimens examined, and types not seen are indicated as such (e.g., MO!, US not seen).
- ☐ Lectotype designations are included together with an indication of where they were designated, the year, and the author. This reference is listed in the Literature Cited. If the author of the paper submitted is making the lectotypification, the phrase "here designated" is used.

8. Tables

- ☐ Tables are neat, double-spaced, and clearly presented. In most cases the printer will typeset these from hard copy.
- ☐ Captions are typed double-spaced as paragraphs at the tops of the tables.
- ☐ Each table starts on a separate sheet.

9. Abbreviations

- ☐ Periods are used after all abbreviations (which are minimized) except metric measures, compass directions, and herbarium designations.
- ☐ When dates are given as part of collection information, three-letter month abbreviations are used, except for months with four letters, which are spelled out in full.
- ☐ States are not abbreviated, and cities are spelled out. [St., as in St. Louis, is acceptable.]
- ☐ Periodicals are abbreviated according to *B-P-H (Botanico-Periodicum-Huntianum)* and to *B-P-H/S (Botanico-Periodicum-Huntianum/Supplementum)*.
- ☐ Authors' names are abbreviated according to Brummit & Powell's *Authors of Plant Names*.
- ☐ Book titles are abbreviated according to *Taxonomic Literature*, edition 2, but with initial letters capitalized. Book titles are spelled out in the Literature Cited.
- ☐ If an item does not appear in *B-P-H* or *TL-2*, or if these references are not available, its title is fully spelled out.
- ☐ Herbaria are abbreviated according to *Index Herbariorum*, edition eight (8).
- ☐ Abbreviated forms are not used for references in the text, except when citing the names of plants. If it is necessary to cite a particular page in the text, the form Smith (1998: 12) is used.

10. Specimens Examined

- ☐ If many specimens were examined, those cited in the text are limited to ca. 1½ manuscript pages.
- ☐ If there are a large number, an index to specimens examined is placed at the end of the paper, following the Literature Cited. It is arranged alphabetically by collector, followed by collection number, followed by the number of the taxon in the text. Names (including initial(s)) of first and second collector are provided, "et al." if three or more.
- ☐ Specimens are cited in the text as follows: *Additional specimens examined (or Selected specimens examined).* MEXICO. **Oaxaca**: Sierra San Pedro Nolesco, Talea, 12°37′N, 85°14′W, 950–1100 m, 3 Feb. 1987 (fl), *Jergensen 865* (BM, G, K, US). [Dates and reproductive status are optional but are omitted from longer lists.] Countries are run together in the same paragraph, e.g., COUNTRY A. **Major political division:** . . . COUNTRY B. **Major political division:** . . . Separate paragraphs are used for major continental regions within major political divisions.

11. Specimen Vouchers and Genetic Sequences

- ☐ If paper presents original data, associated herbarium vouchers are cited. [Vouchers for seed and/or other collections should be included where pertinent. Dependent on the paper, reference to the original wild source may be required.] Vouchers are also cited for common names and uses taken from specimen labels.
- ☐ Herbarium vouchers state the collector and number, herbarium in which the voucher is located, and a clear annotation that the material represents the voucher for the study in question.
- ☐ Nucleic acid or protein sequences corresponding to

equal or greater than 50 nucleotides are entered into an appropriate data bank, e.g., GenBank/EMBL. The accession numbers are provided before publication. [Long sequences (exceeding two pages) will not be routinely published.]

☐ Author accepts responsibility for establishing the accuracy of information provided.

12. Keys

☐ Keys are clear and have been checked carefully for consistency with the descriptions. Leads of each couplet are parallel.
☐ Dichotomous keys are indented.
☐ Infraspecific taxa are keyed separately, not in species keys.

13. Literature Cited

☐ The Literature Cited contains all references cited in the text.
☐ All entries in the Literature Cited are cited in the text.
☐ Spelling of author(s) name(s) and years of publication have been double-checked.
☐ All entries have been verified against original sources, especially journal titles, accents, diacritical marks, and spelling in languages other than English.
☐ Periodicals are listed as follows: author's last name, initial(s). Year. Full title of article. Journal abbreviated as in *B-P-H/S*. Volume: pages. No parenthetical part numbers after volume numbers are given unless each part is paginated separately.
☐ For more than one author, this style is followed: author's last name, initial(s), second author's initial(s), last name & third author's initial(s), last name.
☐ Books appear as follows: author's last name, initial(s). Year. Full Unabbreviated Title (edited by Editor), 3rd ed., Vol. 2. Publisher, City of Publication.
☐ Citations of work "in prep.," unpublished theses and dissertations, and similar references to inaccessible sources have been eliminated or kept to a minimum. They are not necessarily included in the Literature Cited.

14. Illustrations

☐ Author(s) name(s) and figure number(s) are written **in pencil** on the back of each figure or plate, on both originals and review copies.
☐ Scale bars appear on illustrations, photographs, and maps.
☐ Magnifications/reductions are not indicated in captions.
☐ High-quality photocopies of the figures are included with each copy of the manuscript.
☐ Illustrations are presized to fit either column width (2 5/8 in. or ca. 68 mm) or full page width (5½ in. or ca. 140 mm), or illustrations no larger than 11 × 16 in. (= 29 × 40 cm). [Maximum size for printed illustrations is 5½ × 8¼ in.] Oversized or delicate figures are submitted as photostats.
☐ Figures are numbered in Arabic numerals in the order of their citation in the text. Parts of figures are labeled with capital letters.
☐ Photographs are crisp black-and-white prints.
☐ Figures are grouped into composite plates when possible; edges of photographs are abutted.
☐ No stripping is inserted between plate or figure segments. [Printer will insert stripping.]
☐ Edges of figures are squared.
☐ Maps include reference to latitude and longitude and are bounded by a fine border.
☐ Scanning electron micrographs are free of conspicuous charging.
☐ Axes on graphs are all labeled.
☐ Captions provide all explanatory text. Captions are separate from other text, one paragraph for each group of figures, and following the style in current issues of the *Annals*.
☐ Symbols on maps are legible and reduction has been taken into consideration.

Electronic Artwork—Printer's Specifications

1. We accept line art, halftones and color figures on disk or via electronic delivery. Please include a printout or email of the file directory that includes the file name(s), size and kind of file.
2. You should discuss figure sizing specifications with the journal prior to disk submission, and the figures on disk should be the final size desired.
3. Graphic files should be submitted in TIFF, EPS, or PDF file format. Applications that are acceptable are Adobe Photoshop, Corel Draw, Adobe Illustrator and Adobe Freehand, but please, if possible, "Save As" a tif, eps, or pdf file.
1. All line art should be scanned at 1200 d.p.i. All grayscale and color images should be scanned at 350 d.p.i. Color images should be submitted in CMYK mode, not RGB or Indexed Color mode.
5. Include the screen and printer fonts used within the graphic file. Use only Adobe Type 1 postscript fonts. True Type fonts and System "bitmap" fonts should not be used.
6. If the files have been compressed, please indicate what compression program has been used.
7. The disk submission should be accompanied by a hard copy of the figure that exactly matches what is on the disk in the event that the disk is unusable.

Acceptable Electronic Artwork Media

3½ in. floppy disks; Syquest cartridges; EZ Flyer; ZIP disks; JAZ disks; Magneto Optical disks; CD ROM. Artwork may also be submitted via email attachment or FTP. Please contact Karen Ridgway, e-mail address: kridgway@allenpress.com, for more information.

Volume 88, Number 4, pp. 517–737 of the Annals of the Missouri Botanical Garden
was published on January 3, 2002.

ANNALS OF THE MISSOURI BOTANICAL GARDEN

VOLUME 88
2001

Colophon

This volume of the ANNALS of the Missouri Botanical Garden has been set in APS Bodoni. The text is set in 9 point type while the figure legends and literature cited sections are set in 8 point type.

This volume has been printed on 70# Vintage Gloss. This is an acid-free paper designed to have a shelf-life of over 100 years. Vintage Gloss is manufactured by the Potlatch Paper Company.

Photographs used in the ANNALS are reproduced using 300 line screen halftones. The binding used in the production of the ANNALS is a proprietary method known as Permanent Binding.

The ANNALS is printed and distributed by Allen Press, Inc. of Lawrence, Kansas 66044, U.S.A.

© Missouri Botanical Garden 2001

ISSN 0026-6493

VOLUME 88
2001

ALBACH, DIRK C., PAMELA S. SOLTIS, DOUGLAS E. SOLTIS & RICHARD G. OLMSTEAD. Phylogenetic Analysis of Asteridae Based on Sequences of Four Genes 163

ALBERS, FOCKE & ULRICH MEVE. A Karyological Survey of Asclepiadoideae, Periplocoideae, and Secamonoideae, and Evolutionary Considerations within Apocynaceae s.l. 624

ALBERT VICTOR A. (see Kurt Potgieter & Victor A. Albert) 523

ARROYO, MARY K. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo) 669

BERENBAUM, MAY R. Chemical Mediation of Coevolution: Phylogenetic Evidence for Apiaceae and Associates 45

BERNHARDT, PETER. (see Peter Goldblatt, John C. Manning & Peter Bernhardt) 713

BURKE, MICHELE K. (see Gregory M. Plunkett, Porter P. Lowry II & Michele K. Burke 213

CHANDERBALI, ANDRÉ S., HENK VAN DER WERFF & SUSANNE S. RENNER. Phylogeny and Historical Biogeography of Lauraceae: Evidence from the Chloroplast and Nuclear Genomes 104

CIVEYREL, LAURE & NICK ROWE. Phylogenetic Relationships of Secamonoideae Based on the Plastid Gene *mat*K, Morphology, and Biomechanics 583

CRAVEN, K. D., P. T. W. HSIAU, A. LEUCHTMANN, W. HOLLIN & C. L. SCHARDL. Multigene Phylogeny of *Epichloë* Species, Fungal Symbionts of Grasses 14

CROSBY, MARSHALL R. Statistical Summary of Some of the Activities in the Missouri Botanical Garden Herbarium, 2000 159

DAVIDSE, GERRIT. (see Tarciso S. Filgueiras, Gerrit Davidse, Fernando O. Zuloaga & Osvaldo Morrone) 351

DOWNIE, STEPHEN R. (see Krzysztof Spalik & Stephen R. Downie) 270

ENDRESS, MARY E. & W. DOUGLAS STEVENS. The Renaissance of the Apocynaceae s.l.: Recent Advances in Systematics, Phylogeny, and Evolution: Introduction 517

FILGUEIRAS, TARCISO S., GERRIT DAVIDSE, FERNANDO O. ZULOAGA & OSVALDO MORRONE. The Establishment of the New Genus *Altoparadisium* and a Reevaluation of *Arthropogon* (Poaceae, Paniceae) 351

FISHBEIN, MARK. Evolutionary Innovation and Diversification in the Flowers of Asclepiadaceae 603

FISHBEIN, MARK. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo) 669

FORTUNATO, RENÉE H. (see Juan C. Gamerro & Renée H. Fortunato) 144

GAMERRO, JUAN C. & RENÉE H. FORTUNATO. Morfología del Polen de las Especies Argentinas de *Bauhinia* (Cercideae, Caesalpinioideae, Fabaceae) 144

GLENNY, DAVID. (see Peter J. Lockhart, Patricia A. McLenachan, David Havell, David Glenny, Daniel Huson & Uwe Jensen) 458

GOLDBLATT, PETER, JOHN C. MANNING & PETER BERNHARDT. Radiation of Pollination Systems in *Gladiolus* (Iridaceae: Crocoideae) in Southern Africa 713

GRASS PHYLOGENY WORKING GROUP. Phylogeny and Subfamilial Classification of the Grasses (Poaceae) 373

HALLING, ROY. E. Ectomycorrhizae: Co-evolution, Significance, and Biogeography 5

HAVELL, DAVID. (see Peter J. Lockhart, Patricia A. McLenachan, David Havell, David Glenny, Daniel Huson & Uwe Jensen) 458

HOLLIN, W. (see K. D. Craven, P. T. W. Hsiau, A. Leuchtmann, W. Hollin & C. L. Schardl) 14

HOLLOWELL, VICTORIA C. & P. MICK RICHARDSON. Coevolution: Biological Observations from Yucca Moths to Molecules, An Introduction 1

HSIAU, P. T. W. (see K. D. Craven, P. T. W. Hsiau, A. Leuchtmann, W. Hollin & C. L. Schardl) 14

HUFFORD, LARRY. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo 669

HUSON, DANIEL. (see Peter J. Lockhart, Patricia A. McLenachan, David Havell, David Glenny, Daniel Huson & Uwe Jensen 458

JENSEN, UWE. (see Peter J. Lockhart, Patricia A. McLenachan, David Havell, David Glenny, Daniel Huson & Uwe Jensen) 458

KOONTZ, JASON. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo) 669

KUZOFF, ROBERT K. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo) 669

LEUCHTMANN A. (see K. D. Craven, P. T. W. Hsiau, A. Leuchtmann, W. Hollin & C. L. Schardl) 14

LIEDE, SIGRID. Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) Reconsidered: New Evidence Based on cpDNA Spacers 657

LOCKHART, PETER J., PATRICIA A. MCLENACHAN, DAVID HAVELL, DAVID GLENNY, DANIEL HUSON & UWE JENSEN. Phylogeny, Radiation, and Transoceanic Dispersal of New Zealand Alpine Buttercups: Molecular Evidence under Split Decomposition 458

LOWRY, PORTER P. II. (see Gregory M. Plunkett, Porter P. Lowry II & Michele K. Burke) 213

MCLENACHAN, PATRICIA A. (see Peter J. Lockhart, Patricia A. McLenachan, David Havell, David Glenny, Daniel Huson & Uwe Jensen) 458

MANNING, JOHN C. (see Peter Goldblatt, John C. Manning & Peter Bernhardt) 713

MANOS, PAUL S. & DONALD E. STONE. Evolution, Phylogeny, and Systematics of the Juglandaceae 231

MEVE, ULRICH. (see Focke Albers & Ulrich Meve) 624

MOHAMED, KAMAL I., LYTTON JOHN MUSSELMAN & CHARLES R. RICHES. The Genus *Striga* (Scrophulariaceae) in Africa 60

MORAN, NANCY A. The Coevolution of Bacterial Endosymbionts and Phloem-feeding Insects 35

MORRONE, OSVALDO. (see Tarciso S. Filgueiras, Gerrit Davidse, Fernando O. Zuloaga & Osvaldo Morrone) 351

MORT, MARK E. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo) 669

MUSSELMAN, LYTTON JOHN. (see Kamal I. Mohamed, Lytton John Musselman & Charles R. Riches) 60

OLMSTEAD, RICHARD G. (see Dirk C. Albach, Pamela S. Soltis, Douglas E. Soltis & Richard G. Olmstead) 163

PLUNKETT, GREGORY M., PORTER P. LOWRY II & MICHELE K. BURKE. The Phylogenetic Status of *Polyscias* (Araliaceae) Based on Nuclear ITS Sequence Data 213

POTGIETER, KURT & VICTOR A. ALBERT. Phylogenetic Relationships within Apocynaceae s.l. Based on *trn*L Intron and *trn*L-F Spacer Sequences and Propagule Characters 523

PRICE, MICHELLE. Revision of *Rhynchostegiopsis* (Leucomiaceae: Musci) 694

RENNER, SUSANNE S. (see André S. Chanderbali, Henk van der Werff & Susanne S. Renner) 104

RICHARDSON, P. MICK. (see Victoria C. Hollowell & P. Mick Richardson) 1

RICHES, CHARLES R. (see Kamal I. Mohamed, Lytton John Musselman & Charles R. Riches) 60

ROWE, NICK. (see Laure Civeyrel & Nick Rowe) 583

SCHARDL, C. L. (see K. D. Craven, P. T. W. Hsiau, A. Leuchtmann, W. Hollin & C. L. Schardl) 14

SMITH, JAMES F. The Phylogenetic Relationships of *Lembocarpus* and *Goyazia* (Gesneriaceae) Based on *ndh*F Sequences 135

SOLTIS, DOUGLAS E. (see Dirk C. Albach, Pamela S. Soltis, Douglas E. Soltis & Richard G. Olmstead) 163

SOLTIS, DOUGLAS E., ROBERT K. KUZOFF, MARK E. MORT, MICHAEL ZANIS, MARK FISHBEIN, LARRY HUFFORD, JASON KOONTZ & MARY K. ARROYO. Elucidating Deep-level Phylogenetic Relationships in Saxifragaceae Using Sequences for Six Chloroplastic and Nuclear DNA Regions 669

SOLTIS, PAMELA S. (see Dirk C. Albach, Pamela S. Soltis, Douglas E. Soltis & Richard G. Olmstead) 163

SPALIK, KRZYSZTOF & STEPHEN R. DOWNIE. The Utility of Morphological Characters for Inferring Phylogeny in Scandiceae Subtribe Scandicinae (Apiaceae) 270

STEVENS, W. DOUGLAS. (see Mary E. Endress & W. Douglas Stevens) 517

STONE, DONALD E. (see Paul S. Manos & Donald E. Stone) 231

TAYLOR, CHARLOTTE M. Overview of the Neotropical Genus *Notopleura* (Rubiaceae: Psychotrieae), with the Description of Some New Species 478

VENTER, H. JOHAN T. (see Rudolf L. Verhoeven & H. Johan T. Venter) 569

VENTER, H. JOHAN T. & RUDOLF L. VERHOEVEN. Diversity and Relationships within the Periplocoideae (Apocynaceae) 550

VERHOEVEN, RUDOLF L. (see H. Johan T. Venter & Rudolf L. Verhoeven) 550

VERHOEVEN, RUDOLF L. & H. JOHAN T. VENTER. Pollen Morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae) 569

WERFF, HENK VAN DER. (see André S. Chanderbali, Henk van der Werff & Susanne S. Renner) 104

WILAND-SZYMAŃSKA, JUSTYNA. The Genus *Hypoxis* (Hypoxidaceae) in Central Africa 302

ZANIS, MICHAEL. (see Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo) 669

ZULOAGA, FERNANDO O. (see Tarciso S. Filgueiras, Gerrit Davidse, Fernando O. Zuloaga & OSVALDO MORRONE) 351

Missouri Botanical Garden Library
3 1753 00305 8952

ANNALS OF THE MISSOURI BOTANICAL GARDEN
AND MISSOURI BOTANICAL GARDEN ANNUAL REPORT
ARE NOW AVAILABLE IN JSTOR!

JSTOR®, a not-for-profit organization, is an important endeavor dedicated to helping the scholarly community take advantage of advances in electronic technologies. The JSTOR Archive makes available the complete backfiles of *Annals of The Missouri Botanical Garden*, *Missouri Botanical Garden Annual Report (1890-1912)*, and more than 100 other scholarly journals to researchers through participating libraries.

JSTOR has recently announced the release of its Ecology & Botany Collection. Developed in cooperation with the Ecological Society of America, the Collection includes the archives of twenty-nine journals, offering online access to more than 1 million pages of important historic literature.

The Ecology & Botany Collection

American Journal of Botany
American Midland Naturalist
American Naturalist
Annals of the Missouri Botanical Garden
Annual Review of Ecology and Systematics
Biotropica
Brittonia
Conservation Biology
Diversity and Distributions
Ecological Applications
Ecological Monographs
Ecology
Evolution
Functional Ecology
Global Ecology and Biogeography Letters
International Journal of Plant Sciences
Journal of Animal Ecology
Journal of Applied Ecology
Journal of Biogeography
Journal of Ecology
Journal of Tropical Ecology
Journal of The Torrey Botanical Society
Limnology and Oceanography
Missouri Botanical Garden Annual Report
New Phytologist
Paleobiology
Quarterly Review of Biology
Systematic Biology
Systematic Botany

Information regarding JSTOR is available at http://www.jstor.org
120 Fifth Avenue, New York, NY 10011

CONTENTS

The Renaissance of the Apocynaceae s.l.: Recent Advances in Systematics, Phylogeny, and Evolution: Introduction ..... *Mary E. Endress & W. Douglas Stevens* 517

Phylogenetic Relationships within Apocynaceae s.l. Based on *trn*L Intron and *trn*L-F Spacer Sequences and Propagule Characters ..... *Kurt Potgieter & Victor A. Albert* 523

Diversity and Relationships within the Periplocoideae (Apocynaceae) ..... *H. Johan T. Venter & Rudolf L. Verhoeven* 550

Pollen Morphology of the Periplocoideae, Secamonoideae, and Asclepiadoideae (Apocynaceae) ..... *Rudolf L. Verhoeven & H. Johan T. Venter* 569

Phylogenetic Relationships of Secamonoideae Based on the Plastid Gene *mat*K, Morphology, and Biomechanics ..... *Laure Civeyrel & Nick Rowe* 583

Evolutionary Innovation and Diversification in the Flowers of Asclepiadaceae ..... *Mark Fishbein* 603

A Karyological Survey of Asclepiadoideae, Periplocoideae, and Secamonoideae, and Evolutionary Considerations within Apocynaceae s.l. ..... *Focke Albers & Ulrich Meve* 624

Subtribe Astephaninae (Apocynaceae–Asclepiadoideae) Reconsidered: New Evidence Based on cpDNA Spacers ..... *Sigrid Liede* 657

Elucidating Deep-level Phylogenetic Relationships in Saxifragaceae Using Sequences for Six Chloroplastic and Nuclear DNA Regions ..... *Douglas E. Soltis, Robert K. Kuzoff, Mark E. Mort, Michael Zanis, Mark Fishbein, Larry Hufford, Jason Koontz & Mary K. Arroyo* 669

Revision of *Rhynchostegiopsis* (Leucomiaceae: Musci) ..... *Michelle Price* 694

Radiation of Pollination Systems in *Gladiolus* (Iridaceae: Crocoideae) in Southern Africa ..... *Peter Goldblatt, John C. Manning & Peter Bernhardt* 713

Checklist for Authors 735

Index to Volume 88

Cover illustration. Top: *Pronuba yuccasella*, female, in the act of gathering pollen from the anthers of *Yucca filamentosa*. Bottom: Pistils of *Y. filamentosa*, showing, from left, a deformed specimen and varying lengths of the stamens. From *Annual Rep. Missouri Bot. Gard.* 1892, The Yucca Moth and Yucca Pollination, by C. V. Riley. See Coevolution, the 46th Annual Systematics Symposium of the Missouri Botanical Garden: Introduction, first issue of this volume.